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ACTIONS OF γ-AMINOBUTYRIC ACID ON RAT SUPRAOPTIC NUCLEUS NEUROSECRETORY NEURONES *IN VITRO*

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SUMMARY

1. Intracellular recordings were obtained from thirty-eight rat supraoptic nucleus (s.o.n.) neurosecretory neurones in perfused hypothalamic explants. Changes in membrane potential and conductance were monitored following application of γ-aminobutyric acid (GABA), and related agonists and antagonists.

2. GABA depressed action potential discharge of all of thirty-five s.o.n. neurones tested and induced either membrane hyperpolarization or depolarization. Neurones that displayed membrane hyperpolarization in response to lower GABA concentrations (30–300 μM) demonstrated a biphasic membrane voltage change with a later depolarizing phase as a response to higher concentrations (up to 3000 μM).

3. GABA (10–3000 μM) induced a prominent concentration-dependent increase in membrane conductance in all neurones. The critical slope for the log–log plot of [GABA] *vs.* GABA-induced membrane conductance was 1·7, indicating co-operativity in the GABA receptor-induced conductance change.

4. Muscimol (0·3–30 μM) potently mimicked all the effects of GABA. Bicuculline (1–100 μM) antagonized the effects of GABA and muscimol in a competitive manner.

5. Glycine and taurine (1–10 mM) had weak effects, although comparatively similar to those of GABA. These actions were blocked both by bicuculline (100 μM) and by strychnine (1 μM). At higher concentrations (> 10 μM), strychnine also antagonized the actions of GABA.

6. In recordings with potassium-acetate-filled micropipettes, the reversal potential of hyperpolarizing membrane voltage responses to GABA was $-72{\cdot}5 \pm 1{\cdot}5$ mV in close agreement (±5 mV) with the reversal potential of inhibitory post-synaptic potentials (i.p.s.p.s) recorded in the same neurones. Depolarizing responses to GABA reversed polarity at $-50 \pm 1{\cdot}6$ mV. In recordings with KCl-filled micropipettes, voltage responses to GABA were always depolarizing and reversed near $-40{\cdot}0 \pm 4{\cdot}3$ mV. Similarly, reduction of the concentration of chloride ions in the perfusion medium from 134 to 10·4 mM induced a positive shift of the GABA reversal potential by 40–50 mV.

7. From measurements of input resistance (R_{in}) and cell time constant (τ_0), input capacitance (C_{in}; representing total membrane capacitance) was calculated as 78·9

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$\pm 2{\cdot}1$ pF. During responses to GABA or muscimol, decreased R_{in} was accompanied by a linearly related decrease in τ_0 indicating that these substances had no effect on the membrane capacitance of s.o.n. neurones.

8. These results indicate that the actions of exogenously applied GABA on s.o.n. neurones resemble those of spontaneous or evoked i.p.s.p.s in these neurones and involve the activation of a chloride ionic conductance. It is proposed that a majority of the spontaneous and evoked i.p.s.p.s observed in s.o.n. neurones are mediated by GABA acting on $GABA_A$ receptors.

INTRODUCTION

Both intrinsic and synaptic factors are now known to participate in regulating the excitability of hypothalamic supraoptic nucleus (s.o.n.) neurosecretory neurones (for reviews see Poulain & Wakerley, 1982; Renaud, Bourque, Day, Ferguson & Randle, 1985). Recent *in vitro* studies have reported several intrinsic voltage-dependent conductances that participate in osmotically-induced depolarizations (Mason, 1980), and post-spike hyperpolarizing and depolarizing after-potentials (Andrew & Dudek, 1983; Bourque, Randle & Renaud, 1985). *In vivo* extracellular data have characterized the function of different synaptic inputs, and the responses of s.o.n. neurones to a variety of putative neurotransmitters (Poulain & Wakerley, 1982; Renaud *et al.* 1985). Amongst the depressant influences, there is general agreement that the firing of s.o.n. neurones is reduced by activation of peripheral baroreceptors (Harris, 1979), electrical stimulation in limbic brain regions (Cirino & Renaud, 1985) and application of γ-aminobutyrate, or GABA (Nicoll & Barker, 1971; Sakai, Marks, George & Koestner, 1974; Bioulac, Gaffori, Harris & Vincent, 1978; Arnauld, Cirino, Layton & Renaud, 1983). There are additional reasons that now enhance the possibility that GABA has a major regulatory influence in the s.o.n. First is the biochemical (Tappaz, Brownstein & Kopin, 1977; Meyer, Oertel & Brownstein, 1980) and immunohistochemical (Perez de la Mora, Porrani, Tapia, Teran, Palacios, Fuxe, Hokfelt & Ljungdahl, 1981; Tappaz, Wassef, Oertel, Paut & Pujol, 1983; Van den Pol, 1985; Theodosis, Paut & Tappaz, 1986) evidence of its presence within s.o.n. Secondly is the data indicating involvement of forebrain GABAergic mechanisms in neurosecretory cell function (Wible, Zerbe & Di Micco, 1985), in particular to mediate a baroreceptor-activated inhibitory input to s.o.n. vasopressin-secreting neurones (Jhamandas & Renaud, 1986). Moreover, intracellular recordings in perfused hypothalamic explants reveal that s.o.n. neurones receive bicuculline-sensitive, chloride-mediated inhibitory post-synaptic potentials arising from the region of the diagonal band of Broca (Randle, Bourque & Renaud, 1986*b*). We now report the actions of GABA on membrane potential and conductance of s.o.n. neurones *in vitro*.

METHODS

Basal hypothalamic explants measuring approximately $8 \times 8 \times 3$ mm were removed from the brain of male Sprague–Dawley rats (150–300 g) and pinned to the Sylgard (Corning Ltd) base of a perfusion chamber as previously described (Bourque & Renaud, 1983). A glass pipette with a tip diameter of 100–150 μm was positioned in the right internal carotid artery and delivered oxygenated (95% O_2:5% CO_2) and temperature-controlled (34 °C) medium to the explant by

gravity at a rate of 0·6–1·2 ml/min. Osmolality of the medium (296 ± 2 mosM/l) was determined by freezing-point osmometry (Advanced Instruments Microosmometer Model 3MO).

The medium (pH 7·4) contained (in mM): NaCl (126), KCl (4·3), $MgCl_2$ (1·3), $CaCl_2$ (2·4), $NaHCO_3$ (25·9) and glucose (10). Modifications of the ionic composition of the medium included the following: equimolar replacement of NaCl with $MgCl_2.6H_2O$ to obtain 15 mM-Mg^{2+}; replacement of $CaCl_2.2H_2O$ with NaCl to obtain a low-Ca^{2+} medium; equimolar replacement of KCl with sodium glucuronate (Sigma) to obtain 10·4–78 mM-Cl^-.

Media of different compositions were stored in separate reservoirs and selected by remote-controlled solenoid valves. Pharmacological agents dissolved in 200–500 ml of medium to the desired concentrations (see Results) and administered in the same way included tetrodotoxin (TTX, Sigma), bicuculline methiodide (BMI, Sigma) and strychnine sulphate (SSO_4, Sigma). Concentrated solutions (0·1–10 mM) of γ-aminobutyric acid (GABA), glycine, taurine and muscimol (all from Sigma) were injected with a Hamilton syringe pump into the perfusion line at a rate of 2–600 μl/min so as to attain maximum drug concentrations of 0·1 μM to 10 mM.

Intracellular recordings were obtained through micropipettes (impedance range of 100–300 MΩ) filled with 3 M-potassium acetate or potassium chloride (KCl) as specified in the text and connected via a silver–silver chloride electrode to the input stage of a Mentor N-950 preamplifier. Signals were low-pass filtered at 5 kHz and recorded on an FM recorder (Racal Ltd). Analysis of the voltage dependence of the membrane potential changes induced by the pharmacological agents was accomplished by continuous intracellular current injection through a bridge circuit. Periodic (0·5–1 Hz) application of square current pulses (150–200 ms duration, 70–150 pA) allowed estimation of input resistance (R_{in}) and membrane time constant (τ_M). Input conductance (G_{in}) was estimated as a reciprocal of R_{in}. Membrane conductance induced by GABA (ΔG_{in}) and other agents was estimated according to: $\Delta G_{in} = G_{tot} - G_{in}$ (cf. Ginsborg, 1973; Choi & Fischbach, 1981) where G_{tot} represents the input conductance measured at the peak of the response to GABA.

Experiments also evaluated and compared GABA-activated conductances with those evoked synaptically. As detailed elsewhere (see Randle *et al.* 1986*b*) inhibitory post-synaptic potentials (i.p.s.p.s) were evoked in s.o.n. neurones by pulses of electrical stimulation (50–500 μA intensity, 50–200 μs duration) applied to the ventral surface of the diagonal band of Broca through a concentric bipolar electrode (0·3 mm outer diameter).

RESULTS

Data were obtained from a total of thirty-eight s.o.n. neurones with resting membrane potentials more negative than −50 mV, spike amplitudes above 70 mV and membrane input resistances between 94 and 400 MΩ (mean 253 MΩ).

Effects of GABA and muscimol

GABA was added to the perfusion medium so as to attain concentrations of 8 μM to 3 mM for periods of 3–30 s. Data from thirty-five s.o.n. neurones indicate that GABA consistently depressed spontaneous firing in association with membrane potential and conductance changes. In twenty cells where the recording electrode was filled with potassium acetate, the initial membrane response to lower GABA concentrations (30–300 μM) was a hyperpolarization followed by a depolarization at higher GABA concentrations (Figs. 1*A*, 2*A*, 4*A*, 7*A* and 7*B*). In ten other cells recorded with potassium-acetate-filled electrodes, and five cells recorded with KCl-filled electrodes, the initial response was always membrane depolarization (Figs. 1*B*, 5*A*, 8*A*, 9*A* and 10*A*). Either response persisted in the presence of media containing tetrodotoxin (10^{-6} M, Fig. 1*B*), 15 mM-Mg^{2+} or low Ca^{2+}, indicating that GABA acted directly on the post-synaptic membrane of s.o.n. neurones.

In all neurones, GABA induced prominent dose-related reductions in their input

resistance (R_{in}). However, the range of sensitivity to GABA varied widely. Among the 60 % of cells sensitive to 10–100 μM-GABA, a 90–100 % decrease in their R_{in} could be achieved with higher concentrations of GABA (Fig. 2*A*). The remainder were sensitive to 0·1–1 mM-GABA, and reduced their R_{in} by 50–80 % in response to higher GABA concentrations (Figs. 4 and 5). These changes in R_{in} corresponded to GABA-induced conductances (ΔG_{in}) of 10–50 nS (mean 42 nS; see Methods). Despite

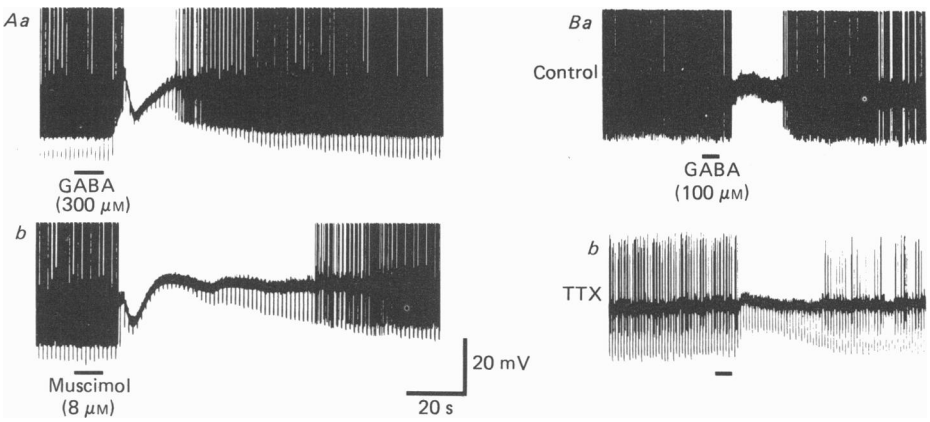

Fig. 1. Intracellular recordings illustrate the responses of two s.o.n. neurones to GABA and muscimol. In this and subsequent Figures, substances were administered into the perfusion line during the interval depicted by the horizontal bars, so as to attain the indicated concentrations. Except where indicated, data were obtained with potassium-acetate-filled micropipettes. Hyperpolarizing current pulses were applied at regular intervals to monitor membrane input impedance. The full amplitude of action potentials is not shown. In *A*, both GABA (*a*) and muscimol (*b*) depress action potential firing while inducing a membrane hyperpolarization and a decrease in input resistance. Note that muscimol's action is more prolonged. Data in *B* is from another cell. In *Ba* the suppressant action of GABA on action potential firing is accompanied by membrane depolarization. Trace *b* illustrates that both actions persist in the presence of tetrodotoxin (TTX, 10^{-6} M).

their differing sensitivities to GABA, all cells had sigmoidal dose–response relationships (ΔG_{in} *vs.* log [GABA]; Figs. 3–6). When data from twenty-nine cells were pooled and log–log plots of the dose–response relationship were constructed (Fig. 3*B*), a critical slope of 1·7 was observed for the response to GABA. However, log–log plots of the dose–response relationships in individual neurones (e.g. Fig. 4*D*) yielded a mean critical slope of $1{\cdot}94 \pm 0{\cdot}21$ ($n = 13$). The reduced slope obtained from plots of pooled data probably results from the variability among neurones in the effective dose of GABA. Nevertheless, these results indicate that there is co-operativity between the binding of two molecules of GABA in the activation of the membrane conductance.

Muscimol, a potent $GABA_A$ receptor agonist, induced membrane voltage and conductance changes similar to those of GABA but at much lower concentrations, ranging between 0·3 and 30 μM (Figs. 1*A*, 2 and 3). Responses to muscimol followed the same pattern of membrane voltage and conductance changes, but lasted 2–3 times longer than the responses to GABA. As with GABA, there was considerable variability between cells in the effective dose range, the sign of the membrane voltage response and the amplitude of the maximally evoked membrane conductance

(compare Figs. 2*B* and 5*B*). However, the dose–response relationships (ΔG_{in} *vs.* log [muscimol]) were sigmoidal (Figs. 3*A* and 5*C*) and the critical slope of the log–log plot of data pooled from nine neurones was 1·6 (Fig. 3*B*). The mean critical slope of plots of the dose–response relationships in four individual neurones was 2·03 ± 0·45.

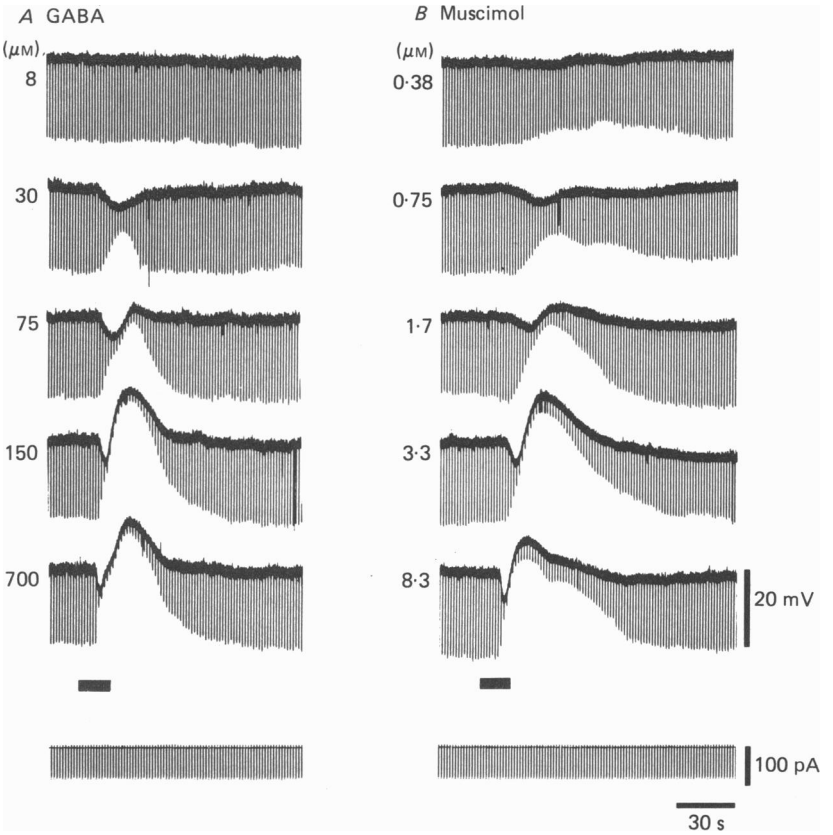

Fig. 2. Chart recordings of the responses of an s.o.n. neurone that was sensitive to low concentrations of GABA and muscimol. The resting membrane potential was −58 to −60 mV throughout; constant-current pulses (70 pA) were administered at 0·5 Hz to monitor input resistance (200–240 MΩ at rest). GABA (*A*) or muscimol (*B*) was administered during the period indicated by the bar at the bottom of each series of records at the concentration indicated to the left of each record. Low doses of both substances induced moderate decreases in input resistance accompanied by a hyperpolarization. Higher doses shunted the membrane resistance and induced a biphasic response where an early hyperpolarization was followed by a late depolarization.

Comparison with taurine and glycine

Two other potential inhibitory neurotransmitters, taurine and glycine, were tested on nine cells. Both substances depressed action potential firing, induced a membrane hyperpolarization or depolarization, and decreased input resistance (Figs. 4*B* and 6*A*). However, their influence on membrane conductance was at least 10-fold less potent than GABA, with maximal GABA-induced conductances between 2- and 5-fold greater than that induced by either taurine or glycine. Furthermore, the

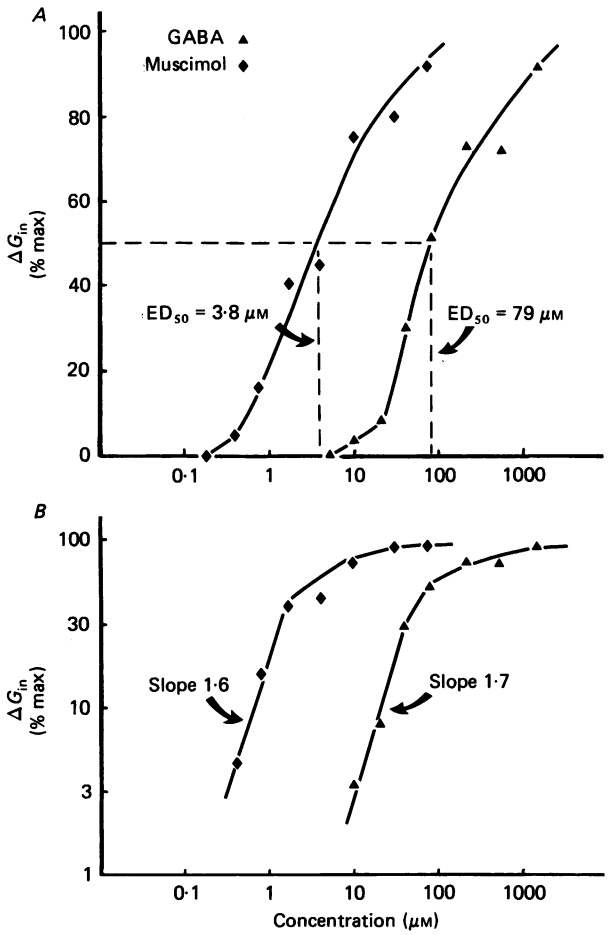

Fig. 3. Dose–response relationships for the increase in input conductance (ΔG_{in}) induced by GABA (triangles) and muscimol (diamonds) in s.o.n. neurones. For individual neurones, conductance changes at each concentration of GABA or muscimol were expressed as a percentage of maximal GABA-induced response in that cell. Data for all cells in this study were then pooled according to the concentration of GABA or muscimol. Each point represents the mean of data obtained from three to eight cells. *A*, semilog plot reveals sigmoidal dose–response relationship. Muscimol was approximately 20-fold more potent than GABA although maximal concentrations of each drug induced identical conductance changes. *B*, log–log plots of the data in *A* have critical slopes of 1·7 (GABA) and 1·6 (muscimol).

limiting slope of the log–log plot of the dose–response relationship (ΔG_{in} *vs.* concentration of drug) was 0·8 for taurine (four cells; cf. Fig. 4*D*) and 1·1 for glycine (three cells), considerably different from GABA and muscimol.

Effects of bicuculline and strychnine

Bicuculline is viewed as a specific $GABA_A$ receptor antagonist in mammalian brain (Curtis, Duggan, Johnston & McLennan, 1971). The dose–response relationships for the conductance induced by GABA were shifted to higher concentrations by

bicuculline methiodide (BMI, 100 μM, thirteen cells), with even more pronounced effects on muscimol responses (Fig. 5). The observation that maximal responses to GABA and muscimol could still be obtained in the presence of bicuculline (Fig. 5) indicates that its inhibition of the actions of GABA and muscimol is competitive in nature. In contrast, bicuculline (100 μM) abolished the responses of three s.o.n. neurones to taurine and glycine.

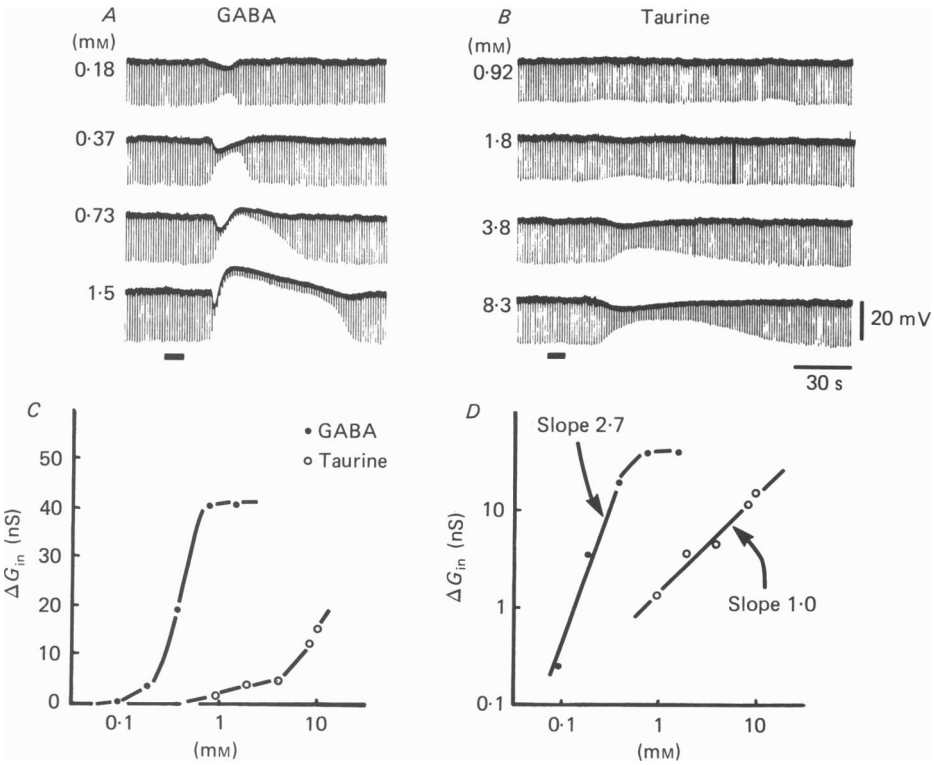

Fig. 4. Comparison of the actions of GABA and taurine on an s.o.n. neurone. GABA (*A*) or taurine (*B*) was administered during the period indicated by the bar at the bottom of each series of records at the concentration indicated to the left of each record. Both substances increased membrane conductance and induced a membrane hyperpolarization. However, high doses of GABA also induced a biphasic effect on membrane potential. The dose–response curve in *C* demonstrates that GABA's effect on membrane conductance was at least 10-fold more potent than taurine. In addition, the log–log plots of their dose–response relationships (*D*) have different slopes: that for GABA is 2·7 while the slope for taurine is 1·0.

While strychnine is viewed as a glycine antagonist, it can, in sufficient concentration, block synaptic transmission mediated by GABA (Choi, Farb & Fischbach, 1981; Scholfield, 1982). Strychnine, at concentrations in excess of 5 μM, can antagonize spontaneous and evoked i.p.s.p.s in s.o.n. neurones (Randle *et al.* 1986*b*). It was therefore deemed important to assess the ability of strychnine to modify responses to exogenous GABA, glycine and taurine. As illustrated in Fig. 6, 1 μM-strychnine abolished the increase in input conductance induced by glycine and taurine but had

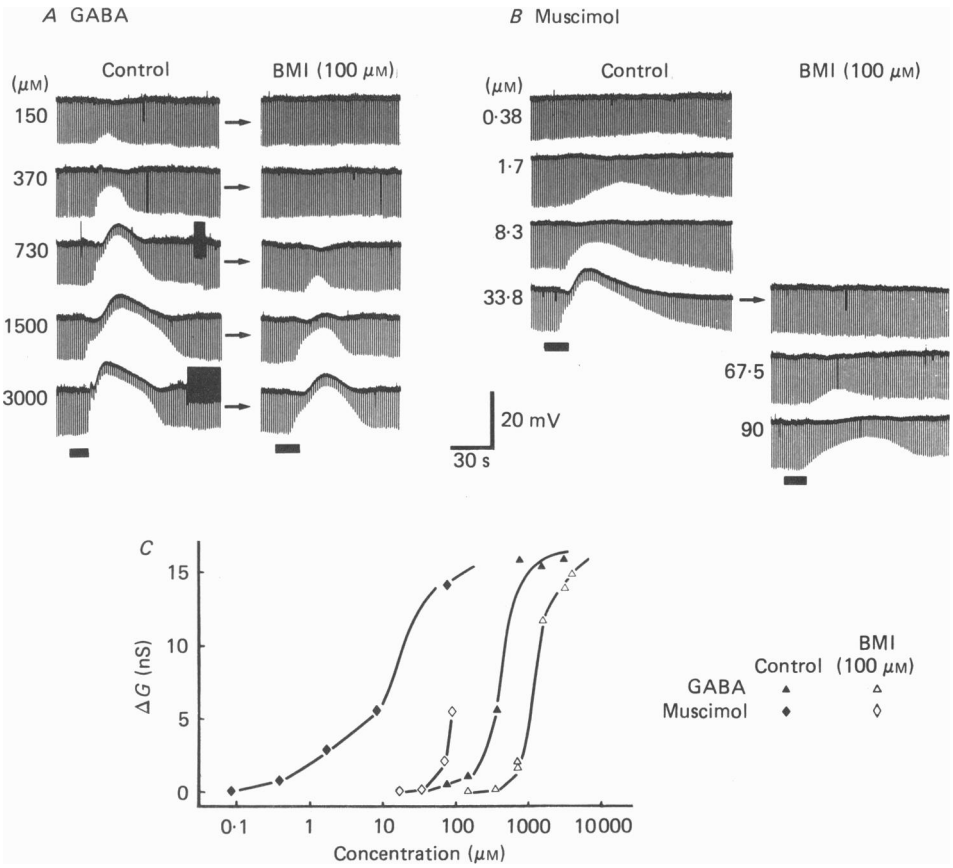

Fig. 5. Antagonism by bicuculline methiodide (BMI, 100 μM) of the effects of GABA (*A*) and muscimol (*B*) on an s.o.n. neurone whose membrane resting potential remained between −62 and −64 mV throughout; 100 pA constant-current pulses indicate that resting input resistance was 240–270 MΩ. Note that for this particular neurone, 5 to 10-fold higher doses of GABA and muscimol were needed to induce increases in input conductance equivalent to those displayed in the cell in Fig. 2. The membrane voltage response was primarily depolarizing. Maximal doses of GABA and muscimol increased input conductance by approximately 15 nS. BMI increased the dose of GABA or muscimol necessary to obtain this maximal response. This is illustrated in *C* where ΔG_{in} is plotted as a function of the concentration of GABA (triangles) or muscimol (diamonds) under either control conditions (filled symbols) or in the presence of BMI (open symbols). The parallel displacement of the dose–response curve for GABA by BMI indicates that BMI acts as a competitive antagonist.

relatively little effect on the response to GABA. In fact, in this and one other s.o.n. neurone, glycine induced a slight decrease in membrane conductance in the presence of strychnine. At higher concentrations (10–100 μM), strychnine also reduced the GABA-induced membrane conductance (four cells). This concentration range corresponds exactly to that which blocks i.p.s.p.s in s.o.n. neurones (Randle *et al.* 1986*b*).

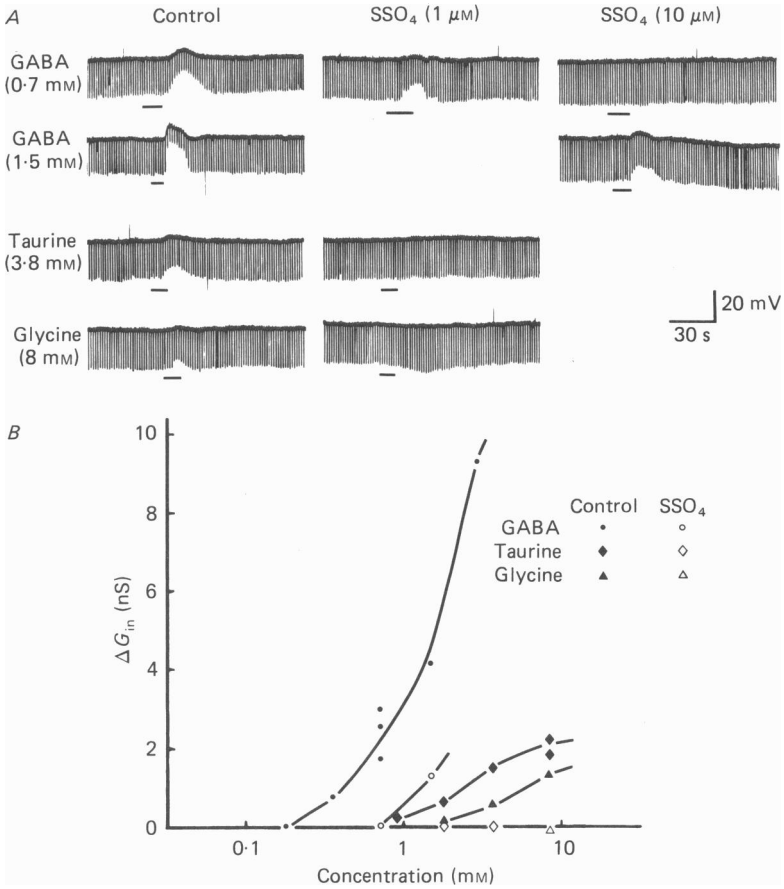

Fig. 6. Antagonism by strychnine sulphate (SSO_4) of the effects of GABA, taurine and glycine on an s.o.n. neurone. *A*, the substances were applied during the periods indicated by the bars at the concentration indicated to the left of each row of records. SSO_4 (1 μM) had comparatively little effect on the response to GABA but abolished the response to taurine and altered the response of glycine such that it now induced a decrease in membrane conductance. Higher concentrations of SSO_4 (10 μM) blocked the response to low doses of GABA and reduced the conductance induced by higher doses. *B*, plots of the membrane conductance induced by GABA (circles), taurine (diamonds) and glycine (triangles) in the absence (filled symbols) or presence (open symbols) of SSO_4.

GABA-activated ionic conductance

The amplitude of the membrane responses to GABA were related to membrane potential in a linear manner. In all of eight cells tested where the initial response to GABA was a membrane hyperpolarization, responses were reduced and eventually reversed by membrane hyperpolarization, with a mean reversal potential (E_{GABA}) of $-72{\cdot}5 \pm 1{\cdot}5$ mV. For these same cells, E_{GABA} was similar (± 5 mV) to the reversal potential of the i.p.s.p., ($E_{i.p.s.p.}$) evoked by electrical stimulation of the ventral surface of the diagonal band of Broca (Fig. 7*C* and *D*; cf. Randle *et al.* 1986*b*). In

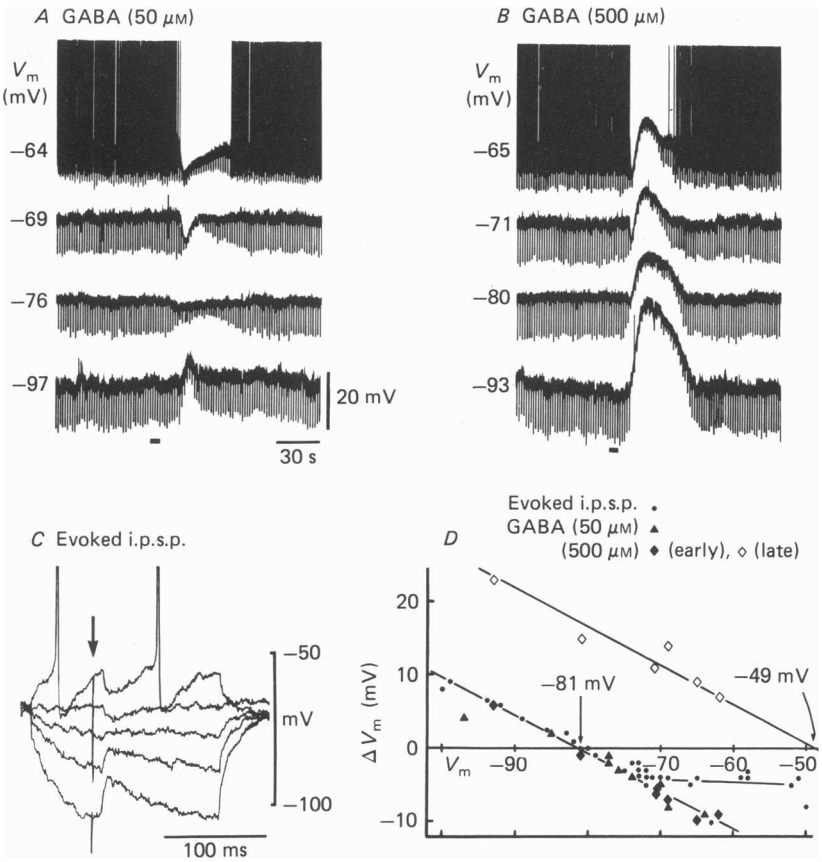

Fig. 7. Relationship between the hyperpolarization and biphasic responses to GABA (*A*, *B*) and the i.p.s.p. (*C*) evoked in the same s.o.n. cell by an electrical stimulus applied in the ventral part of the diagonal band of Broca. GABA was administered during the period indicated by the bar at 50 μM (*A*) or 500 μM (*B*) and evoked hyperpolarizing or biphasic responses, respectively. The membrane potential (V_m indicated to the left of each record) was adjusted by steady intracellular current injection and the sign and amplitude of the membrane voltage responses (ΔV_m) were observed. Similarly in *C* the sign and amplitude of the i.p.s.p. evoked by electrical stimulation (arrow depicts shock artifact) of the diagonal band of Broca (cf. Randle *et al.* 1986*b*) was monitored as the membrane potential was adjusted by intracellular injection of current pulses that were 180 ms in duration. *D*, ΔV_m was plotted as a function of membrane potential. The hyperpolarizing responses to GABA showed a voltage dependence very similar to that of the evoked i.p.s.p. Both responses reversed near −81 mV. The reversal potential of the depolarizing response to GABA was estimated (by extrapolation) to be −49 mV.

contrast, the extrapolated reversal potential for any GABA-evoked membrane depolarizations, whether as initial events or following an initial hyperpolarization, ranged between −33 mV and −55 mV (mean = 50·9 ± 1·6 mV; Figs. 7 and 8).

In each of five cells recorded with KCl-filled micropipettes, GABA-induced changes in membrane conductance were similar to those observed with potassium-acetate-filled electrodes. However, as noted earlier, no GABA-induced membrane hyperpolarizations were noted; voltage responses to GABA were always in the depolarizing

direction, with a mean E_{GABA} of $-40{\cdot}0 \pm 4{\cdot}3$ mV (range -29 to -55 mV; Fig. 9). As illustrated in Fig. 9*C*, E_{GABA} was in close agreement with $E_{i.p.s.p.}$ (± 4 mV).

Since the preceding data suggested that GABA activated a chloride conductance, the effects of altering the extracellular chloride concentration on GABA-induced responses were examined on three neurones recorded with potassium-acetate-filled

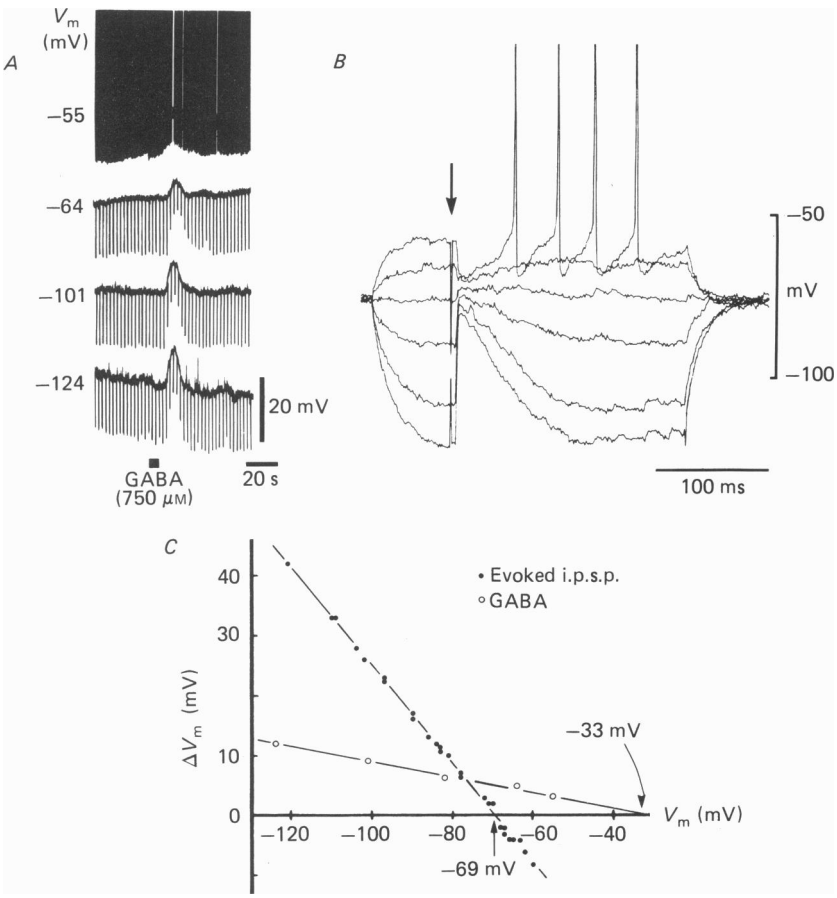

Fig. 8. Relationship between the depolarizing response to GABA (*A*) and the evoked i.p.s.p. (*B*) in an s.o.n. neurone recorded with a potassium electrode. Same analysis as in Fig. 7*C* and *D*. Note in *C* that the reversal potentials of the GABA and i.p.s.p.-induced voltage changes differ by approximately 36 mV.

micropipettes. A reduction in the extracellular chloride concentration from 134 to 10·4 mM induced a positive shift of E_{GABA} by 40–50 mV (Fig. 10), similar to the shift of $E_{i.p.s.p.}$ induced by this manoeuvre (cf. Randle *et al.* 1986*b*). Despite these marked differences in GABA-induced membrane voltage and i.p.s.p.-associated conductances consequent to a reduction in the extracellular chloride concentration, there was no apparent alteration in GABA-induced membrane conductance.

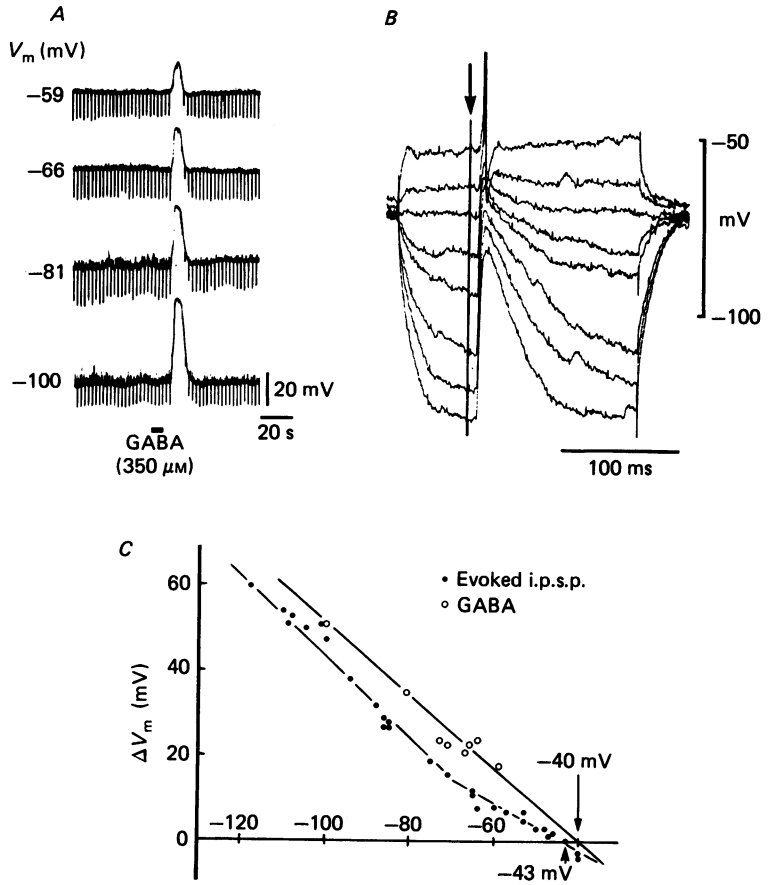

Fig. 9. Relationship between the depolarizing response to GABA (*A*) and the i.p.s.p. (*B*) in an s.o.n. neurone recorded with a KCl-filled electrode. Same analysis as in Fig. 8. Note in *C* that the reversal potentials of GABA and i.p.s.p.-induced voltage responses are similar.

Effect of GABA on cell time constant and input capacitance

Input capacitance (C_{in}) can be approximated from experimentally derived values of the whole cell time constant (τ_o) and input resistance (R_{in}) using the relationship:

$$C_{in} = \tau_o / R_{in}. \quad (1)$$

In s.o.n. neurones, where spread of current between soma and dendrites is effective, these quantities approximate the total membrane capacitance (C_M), the membrane time constant (τ_M) and the total membrane resistance (R_M). Mean C_M calculated in this fashion for twenty-one s.o.n. neurones was 78·9±2·1 pF (±S.E. of mean) and ranged between 29·8 and 214·9 pF.

During responses to GABA and muscimol, the decreased R_{in} is accompanied by a fall in τ_o (Fig. 11) and the relationship between these two variables reflects the status of the C_{in} (according to eqn. (1)). Fig. 11*C* shows that τ_o is linearly related to R_{in}

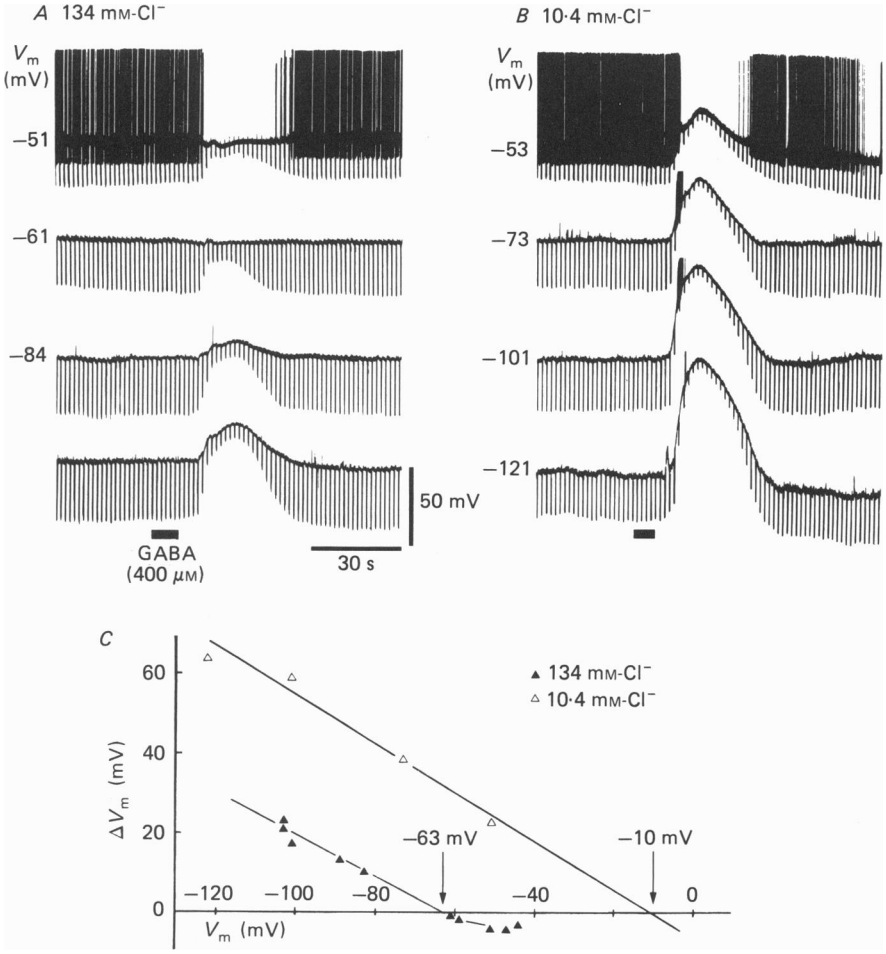

Fig. 10. Dependence of the reversal potential of the GABA-induced voltage responses (E_{GABA}) upon the extracellular concentration of chloride ions. Similar analysis as in Fig. 8. In *A*, responses to GABA were observed in medium containing the normal concentration of chloride (134 mM); in *B*, the medium contained 10·4 mM-Cl^- in which NaCl was replaced by sodium glucuronate. The plot in *C* illustrates that E_{GABA} was shifted from −63 mV to approximately −10 mV by the reduction of extracellular chloride.

indicating that C_{in} (the slope of τ_o *vs.* R_{in} = 42 pF) is unaffected by muscimol. This was true of all six s.o.n. neurones studied in this manner where the mean slope was 57·1 ± 2·6 pF. In four of the six neurones, the relationship was extrapolated through the origin (as in Fig. 11*C*); in the other two cells, the line intersected the abscissa or ordinate, indicating the existence of a second, shorter time constant or a slight bridge imbalance.

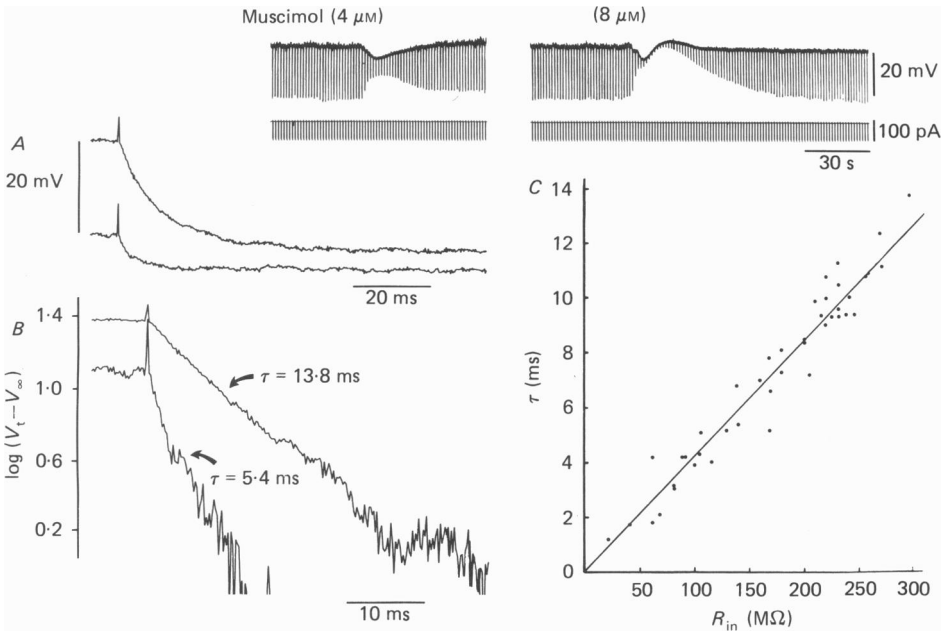

Fig. 11. Lack of effect of muscimol on membrane capacitance as illustrated by the linear relationship between cell time constant (τ) and input resistance (R_{in}). The membrane voltage responses to individual current pulses were analysed over the course of two responses of an s.o.n. neurone to muscimol (4 and 8 μM). In *A*, two pulses are plotted to illustrate the exponential saturation of the membrane voltage response to the square current pulse. The larger response was obtained prior to application of muscimol; the smaller at the peak of the response to 4 μM-muscimol. In *B*, these data are plotted on semilogarithmic coordinates to illustrate the single exponential saturation kinetics. τ was calculated as the time taken for the membrane voltage to decay from $V_t - V_\infty$ to $1/e \times (V_t - V_\infty)$, i.e. 0·56 log units. In *C*, τ calculated in this manner for forty-three current pulses is plotted as a function of R_{in} calculated from the amplitude of the voltage deflexion. The data are well fitted by a straight line having slope 42·3 pF ($r = 0{\cdot}93$) indicating that there is no change in total membrane capacitance in response to muscimol.

DISCUSSION

The principal objective of these experiments was to test the hypothesis that GABA may mediate at least one form of synaptic inhibition, i.e. post-synaptic inhibition, in the s.o.n. For this hypothesis to be true, exogenously applied GABA and synaptically evoked i.p.s.p.s should elicit similar changes in the membrane properties of s.o.n. neurones and should demonstrate similar sensitivity to a specific antagonist (cf. Werman, 1966). In fact, GABA's actions proved to be very similar to those of the i.p.s.p.s. GABA: (1) inhibited action potential firing, (2) increased membrane conductance and (3) induced a hyperpolarization in most neurones which reversed near $E_{i.p.s.p.}$ and was sensitive to the transmembrane chloride gradient. Therefore, similar to the i.p.s.p.s recorded in s.o.n. neurones, the effects of GABA appear to result from the activation of a chloride ionic conductance.

The ability of bicuculline to antagonize the effects of GABA, as it does the i.p.s.p.s, is further evidence for GABA mediation of the i.p.s.p.s, and suggests that the chloride

ionophore is linked to $GABA_A$ type receptors (for review, see Enna & Gallagher, 1983). Biochemical studies suggest that the inhibition by bicuculline of GABA binding to $GABA_A$ receptors is competitive in nature (Enna & Snyder, 1977; Mohler & Okada, 1978). Several electrophysiological studies (Bowery & Brown, 1974; Homma & Rovainen, 1978; Pickles, 1979; Simmonds, 1980) indicate that inhibition of GABA actions by bicuculline resembles competitive inhibition because, in the presence of bicuculline, higher doses of GABA can still evoke maximal responses. This is manifested in the parallel shift of the dose–response curve for GABA induced by bicuculline in s.o.n. neurones (Fig. 5).

We have recently noted (Randle *et al.* 1986*b*) that strychnine may depress evoked i.p.s.p.s in s.o.n. neurones, thereby raising the possibility that glycine or taurine might participate in synaptic inhibition. However, the actions of glycine and taurine in the s.o.n., although similar, appear to be weak relative to those of GABA (Figs. 4 and 6). Furthermore, while concentrations of strychnine in excess of 5 μM are required to inhibit i.p.s.p.s or the actions of GABA, the actions of glycine and taurine are abolished by 1 μM-strychnine (Fig. 6). These results support our proposal (Randle *et al.* 1986*b*) that i.p.s.p.s in s.o.n. neurones are mediated by GABA and that other neutral amino acids such as glycine and taurine are relatively unimportant to synaptic inhibition in the s.o.n.

Effects of muscimol

Muscimol, a powerful $GABA_A$ agonist, was observed to be twenty times more potent than GABA in inducing a chloride conductance in the s.o.n. This potency ratio is similar to that observed in the olfactory cortex (Pickles, 1979; Brown & Galvan, 1979; Simmonds, 1980; Scholfield, 1982) and is further evidence that the effects of GABA on s.o.n. cells are mediated by $GABA_A$ receptors (Enna & Gallagher, 1983). Biochemical studies indicate a smaller (3 to 8-fold) difference in the affinities of GABA and muscimol for the $GABA_A$ receptor (Snodgrass, 1978; Beaumont, Chilton, Yamamura & Enna, 1978; Mohler & Okada, 1978; Williams & Risley, 1979). Although the reason for this discrepancy has not yet been determined, Brown & Galvan (1979) have speculated that GABA is less accessible to the receptor binding site because of glial uptake (cf. Iversen & Kelly, 1975). Muscimol and bicuculline are poor substrates for the sodium-dependent GABA binding site thought to mediate GABA uptake (Johnston, Kennedy & Lodge, 1978). This could explain not only the greater potency of muscimol, but the longer duration of responses compared to GABA (Figs. 1 and 2). The greater sensitivity of muscimol to antagonism by bicuculline methiodide (Fig. 5; see also Simmonds, 1980; Scholfield, 1982) can also be explained on this basis. GABA, at higher concentrations (*vs.* muscimol) necessary to induce a given conductance change, competes more effectively with bicuculline methiodide for $GABA_A$ binding sites.

This discrepancy in potency ratio could also be the result of differing abilities of GABA and muscimol to activate the receptor–ionophore complex following receptor binding. Mathers & Barker (1981) suggested that muscimol activates a channel having a mean open time twice that of the channel activated by GABA. Pertinent to this discussion is the observation that the slope of the log–log plots of ligand-induced currents as a function of ligand concentration is approximately 2 for both

GABA and muscimol (Fig. 3). This value is similar to that observed in several other systems (Barker & Ransom, 1978; Krnjevic, Puil & Werman, 1977; Choi & Fischbach, 1981; Akaike, Hattori, Inomata & Oomura, 1985) and has been held as evidence that two molecules (of either GABA or muscimol) combine with the receptor to activate the chloride ionophore. This similarity of the slopes for GABA and muscimol argues that differences in ionophore activation kinetics do not result from differences in the stoichiometry of the receptor binding–activation sequence for the two agents. A more likely explanation is that the higher affinity of muscimol for the GABA binding site results from slower dissociation of the muscimol–receptor complex. This decreased rate of dissociation might result in slower channel inactivation kinetics.

Biphasic and depolarizing responses to GABA

Perhaps the most perplexing aspect of the results of these experiments is the dichotomy in the data concerning the reversal potential of the membrane voltage response to GABA (E_{GABA}). Approximately 60 % of s.o.n. neurones responded to low doses of GABA (10–100 μM) and E_{GABA} was very similar to the $E_{i.p.s.p.}$. In the remaining neurones, E_{GABA} was 10–36 mV more positive than the $E_{i.p.s.p.}$, and these cells usually responded only to concentrations of GABA greater than 100 μM. No other membrane property could be found that demonstrated a strong correlation with E_{GABA}. In particular, there was no correlation between the strength of the evoked i.p.s.p. in a given neurone, and its responsiveness to GABA.

Biphasic responses to GABA have been reported in other central nervous system neurones (Barker & Ransom, 1978; Andersen, Dingledine, Gjerstad, Langmoen & Laussen, 1980; Alger & Nicoll, 1982; Scharfman & Sarvey, 1985). It appears that hyperpolarizing responses result from chloride influx due to activation of somatic GABA receptors while depolarizations result from activation of receptors located on dendrites. If one assumes that receptors in both sites are associated with chloride channels, then it would appear that the electrochemical chloride gradient across the dendritic plasma membranes is reversed with respect to the somatic plasma membrane. Alternatively, high concentrations of GABA may act on a separate class of receptors which activate a different ionic conductance or, as suggested by Krnjevic *et al.* (1977) electrogenic Na^{2+}-dependent GABA uptake. The latter possibility would appear unlikely since muscimol can also provoke depolarizing responses although it is a poor substrate for active uptake mechanisms (see above). The participation of another ionic conductance is an attractive possibility in that it might also explain the lack of a change in GABA-induced conductance in low-Cl^- medium. At present, however, the data are insufficient to resolve this question.

Membrane capacitance and the effects of GABA

The mean total membrane capacitance (C_m) of twenty-one s.o.n. neurones was found to be $78{\cdot}9 \pm 2{\cdot}1$ pF. Using $1{\cdot}0$ μF/cm^2 as an estimate of specific membrane capacitance, the surface area of an s.o.n. neurone can be estimated as follows:

$$\text{area} = \frac{\text{total membrane capacitance}}{\text{specific membrane capacitance}} = \frac{78{\cdot}9 \times 10^{-12}\ \text{F}}{1{\cdot}0 \times 10^{-6}\ \text{F/cm}^2} = 7890\ \mu\text{m}^2.$$

Using the data of Randle, Bourque & Renaud (1986*a*), an s.o.n. neurone can be

modelled as an elliptical soma of dimensions 15×25 μm with three dendrites of length 300 μm that taper gradually from an initial diameter of 3 μm to a tip diameter of 0·5 μm. The thinner axon can be included as having average diameter of approximately 1·0 μm. Thus, surface area of the neurone equals

$$\text{soma surface} + 3(\text{dendrite surface}) + \text{axonal surface}$$

$$= \pi(15)(25) + 3\left[\pi\left(\frac{3+0{\cdot}5}{2}\right)(300)\right] + \pi(1)(300)\ \mu\text{m}^2$$

$$= 7069\ \mu\text{m}^2.$$

The close agreement of these independent estimates of cell surface area is evidence that current spread into distal dendrites is extremely effective in s.o.n. neurones.

GABA did not alter the total membrane capacitance of s.o.n. neurones. This would indicate that its membrane effects are limited to the receptor ionophore and do not involve membrane lipids.

The powerful effects of GABA on s.o.n. neurones observed in this study lend further support to the emerging role of GABA as an important regulatory agent in the release of neurohypophysial peptides. Central administration of GABA attenuates vasopressin release in response to polyethylene-glycol-induced hypovolemia (Knepel, Nutto & Hertting, 1980), to bilateral carotid occlusion (Feldberg & Roche e Silva, 1981) and to hypertonic saline (Brennan & Haywood, 1985). Emphasis on a forebrain GABAergic mechanism directly involving neurosecretory neurones (cf. Wible *et al.* 1985) is supported by our recent *in vivo* evidence (Jhamandas & Renaud, 1986), that forebrain GABAergic neurones participate in a selective depression of the excitability of s.o.n. vasopressin-secreting neurones consequent to activation of peripheral high-pressure baroreceptors. However, GABA is widely distributed within s.o.n., and appears to influence the excitability of both vasopressin- and oxytocin-secreting neurones (Arnauld *et al.* 1983). Hence, one can anticipate that this amino acid will eventually be linked to additional neural circuits that regulate the excitability of both classes of neurosecretory neurones.

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