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SL 75 102 AS A γ-AMINOBUTYRIC ACID AGONIST: EXPERIMENTS ON DORSAL ROOT GANGLION NEURONES *in vitro*

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1 In anticipation that centrally active γ-aminobutyric acid (GABA)-mimetic drugs may be clinically useful, derivatives of GABA with an imine link (Schiff base) to a lipophilic carrier have recently been prepared. The present paper concerns the actions of [α(4-chlorophenyl)5-fluoro, 2-hydroxy benzilidene-amino]-4-butanoate Na^+, SL 75 102.

2 To test one aspect of the GABA-mimetic properties of SL 75 102, this compound was compared with GABA for activity on intracellularly-recorded neurones in rat dorsal root ganglia *in vitro*. On these neurones GABA, administered either by microiontophoresis or direct into the superfusion medium, causes a depolarization, due to an increased chloride conductance, followed by a period of desensitization.

3 The actions of SL 75 102 were in nearly all respects identical to those of GABA; parameters examined were the effects on membrane potential and input conductance, desensitization, dose-response characteristics and sensitivity to the GABA antagonists, bicuculline and picrotoxin.

4 SL 75 102 was less potent than GABA (mean relative potency 0·03:1).

5 SL 75 102 therefore appears to be a weak agonist at GABA receptors of these neurones.

Introduction

γ-Aminobutyric acid (GABA) is a well established neurotransmitter in the vertebrate central nervous system. A dysfunction of GABA neurones is thought to occur in certain disease such as epilepsy, Huntington's chorea and Parkinson's disease, and it has been suggested that an augmentation of endogenous GABA activity might ameliorate the clinical symptoms of these and other diseases (Meldrum, 1975; Lloyd, 1980). GABA itself does not cross the adult blood–brain barrier, so that in recent years attention has been focused on other GABA agonists which might enter the brain more efficiently. Muscimol raised hopes in this respect, but this potent GABA receptor agonist has recently been shown to enter the brain poorly and to be metabolised rapidly (Baraldi, Grandison & Guidotti, 1979; Maggi & Enna, 1979) so that the behavioural effects which follow its systemic administration (Waser, 1967) may be partly due to transaminated metabolites; this fact may explain the unacceptable toxicity shown by this isoxazole (Chase & Walters, 1976; Chase & Taminga, 1979).

Recently derivatives of GABA have been prepared which have an imine link (Schiff base) to a lipophilic carrier and which readily cross the blood–brain barrier (Lloyd, Worms, Depoortere & Bartholini, 1979). [α(4-Chlorophenyl)5-fluoro, 2-hydroxy benzilidene-amino]-4-butyramide (SL 76 002) and one of its major metabolites, the sodium salt of the 4-butanoic acid derivative (SL 75 102) have recently been synthesized and are undergoing tests to characterize their actions as agonists at mammalian GABA receptors. Both SL 76 002 and SL 75 102 displace [^{3}H]-GABA from membrane binding sites *in vitro* (Bartholini, Scatton, Zivkovic & Lloyd, 1979) and antagonize bicuculline convulsions in mice (Lloyd *et al.*, 1979) and, in preliminary electrophysiological tests, SL 75 102 exhibited GABA-mimetic activity on rat dorsal root ganglion (DRG) neurones *in vitro* (Desarmenien, Headley, Santangelo & Feltz, 1980). Because SL 76 002 is not soluble in aqueous solutions, we have continued to investigate SL 75 102 which is readily soluble; in the present paper we describe more fully the GABA-mimetic activity of SL 75 102 on

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DRG neurones. These cells possess well-characterized receptors for GABA (Deschenes, Feltz & Lamour, 1976; Gallagher, Higashi & Nishi, 1978; Desarmenien, Feltz & Headley, 1979b; 1980). On the other hand, other amino acid neurotransmitters such as glycine and glutamate do not affect the membrane potential or conductance; nor does baclofen (Feltz, Deschenes & Desarmenien, 1978) which has recently been proposed as an agonist at a bicuculline-insensitive GABA receptor (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull, 1980).

Methods

Charles Rivers rats (50 to 120 g) were stunned and killed, and the spinal column was rapidly removed, hemisected and placed in a bicarbonate Ringer at room temperature. Lumbar ganglia, together with their attached spinal roots and peripheral nerve, were dissected out and stored in Ringer solution at room temperature. One ganglion was selected and its ventral root was removed and the nerve ends were tied; the connective tissue sheath was removed with forceps. The ganglion was pinned on Sylgard (Rhône Poulenc, RTV 141) in a simple superfusion chamber (volume 150 to 250 μl) which was then placed on the fixed stage of a conventional-optics microscope. The ganglion was continuously superfused with the Ringer solution (30 to 35°C) at 1 to 3 ml/min. At a final magnification of ×250 or ×320, neurones could be seen clearly and micropipettes could be positioned precisely in relation to any particular cell. Surface neurones were impaled with single barrel micropipettes which were filled with 4 M potassium acetate and had tip resistances of 20 to 60 MΩ. Membrane resistance was measured with a bridge circuit and hyperpolarizing constant current pulses (usually 300 ms, 0.5 to 2.0 nA at 1 Hz). The Ringer solution used in these experiments had the following composition (mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26 and glucose 11. The solution was bubbled continuously with 95% O_2 and 5% CO_2 giving a pH near to 7.4.

Compounds were administered either by addition to the superfusing Ringer solution, or by drop (see below). GABA was often also administered microelectrophoretically from a single barrel pipette which was positioned 10 to 100 μm upstream from the neurone under study.

Whilst satisfactory responses can be elicited by iontophoretic GABA, there remains the problem that the concentration of GABA attained at the neuronal membrane is both unknown and uneven. On the other hand, known concentrations of GABA cannot be administered by superfusion because of the rapid desensitization of DRG neuronal GABA receptors (Desarmenien *et al.*, 1979b; 1980): even with rapid superfusion rates it is not feasible to reach equilibrium concentrations in the recording chamber within the 1 to 3 s latency to the onset of receptor desensitization. We have therefore adopted a system of administration by drop (a constant 5 μl from a Hamilton syringe) direct into the recording chamber. The effective dilution of this drop of concentrated solution in the superfusion Ringer could be estimated for each cell in the following way. The plateau depolarization resulting from the addition of 6 to 9 mM KCl to the superfusion medium was compared with the transient depolarization evoked by drops of various more concentrated solutions of KCl. The relative concentrations eliciting similar amplitude depolarizations provided a dilution factor, which in these experiments ranged from 5 to 100, depending on the rate of superfusion and on the position in the chamber at which the drops were placed. The constancy of responses administered by drop was, in most experiments, good ($< \pm 10\%$). Any variations due to the administration technique could be distinguished from changes in cell sensitivity by comparison of GABA responses evoked by drop with those elicited iontophoretically. We have assumed that the dilution factor will apply approximately equally to different concentrations of drugs and different compounds, an assumption which seems justified since all recordings were made from the surface layer of cells (see also Discussion). All concentrations referred to in the results section are to the estimated final concentration.

The compounds used in these experiments were as follows. GABA (Sigma) 1 M, pH 3 in iontophoretic pipettes or 10^{-5} to 10^{-2} M in 165 mM NaCl by drop; SL 75 102 (provided by Dr K.G. Lloyd of Synthelabo, Paris) 10^{-4} to 10^{-1} M by drop; bicuculline (Pierce) and picrotoxin (Sigma) 10^{-6} to 10^{-5} M by superfusion. The structure of SL 75 102 is illustrated in Figure 1.

The sodium salt of SL 75 102 dissociates slowly in aqueous solutions and free GABA is liberated (Lloyd,

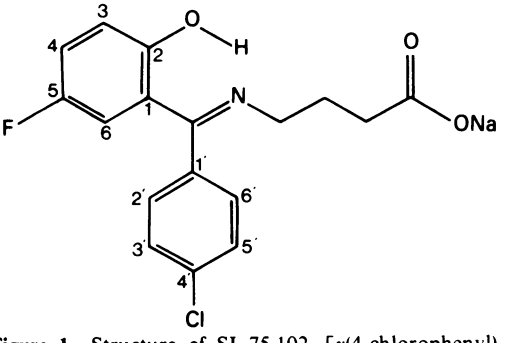

Figure 1 Structure of SL 75 102, [α(4-chlorophenyl)-5-fluro, 2-hydroxy benzilidene-amino]-4-butanoate sodium.

personal communication). In all experiments we have therefore tested solutions prepared within one hour of use. Even after this relatively short time interval there was sometimes an apparent increase in the potency of SL 75 102, an observation which is explicable in terms of the small (1 to 5%) release of free GABA at one hour.

Results

Responses to GABA

Drop administrations of GABA evoked depolarizing responses in rat DRG neurones resembling those obtained by superfusion or iontophoresis *in vivo* or *in vitro* (Desarmenien *et al.*, 1979b; 1980). The depolarization was accompanied by an increase in conductance, as measured by a reduction of the voltage steps evoked by constant current hyperpolarizing pulses. In the example in Figure 2a the bridge system was balanced after penetration of the cell, so that the reduced hyperpolarizing voltage steps are seen as depolarizing voltage steps (depolarization upwards in Figures 2, 3 and 5). That these responses were caused by activation of GABA receptors is indicated by the following criteria: (i) the responses were sensitive to the GABA antagonists, bicuculline and picrotoxin (1 to 5 μM; see also below), (ii) successive GABA administrations, whether by drop or by iontophoresis, elicited progressively smaller responses, a manifestation of the rapid desensitization of GABA receptors which occurs at this site (Figure 2b; see also Desarmenien *et al.*, 1979b; 1980), (iii) drops of water, 165 mM NaCl or Ringer solution elicited only small (<2 mV) membrane potential changes with virtually undetectable changes of membrane input resistance.

Responses to SL 75 102

SL 75 102 was tested by drop on a total of 72 DRG neurones all of which were shown to be sensitive to drop administrations of GABA. On all cells SL 75 102 (solutions prepared 2 to 60 min previously) evoked responses similar to those elicited by GABA, i.e. depolarization and (on the 45 cells so tested) increased membrane conductance (Figure 2a). The time course of the responses was similar to that of GABA re-

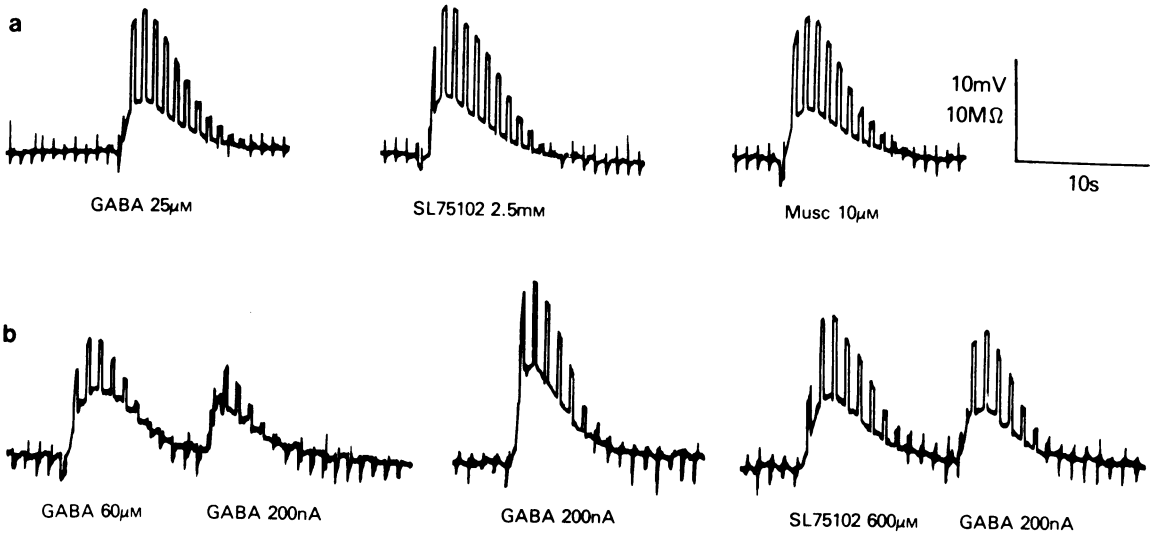

Figure 2 Comparison of responses to γ-aminobutyric acid (GABA) and to SL 75 102 on dorsal root ganglion (DRG) neurones *in vitro*. (a) Responses evoked by GABA 25 μM, SL 75 102 2.5 mM and muscimol 10 μM. Note similarity of response characteristics but difference in potency. (b) SL 75 102, like GABA, desensitizes GABA receptors: here the responses to iontophoretic GABA (200 nA, 1 s; control in centre) are reduced by GABA (left) or SL 75 102 (right) administered 12 and 10 s beforehand, respectively. Administration either by iontophoresis [GABA test responses in (b)] or by drop direct into the recording chamber. Values given are final concentrations estimated as described in Methods. In (a) the cell's resting potential was −66 mV and resting input resistance was 17 MΩ. The hyperpolarizing voltage steps evoked by constant current hyperpolarizing pulses (1.0 nA, 300 ms at 1 Hz) were balanced by the bridge circuit after penetration of the cell, so that the upward deflections seen represent a reduction of input resistance. In (b) the resting potential was −61 mV and resting input resistance was 22 MΩ. Current pulses as in (a).

sponses; thus the time from peak to half decay was the same as for GABA, although the time to peak was consistently slightly shorter. However, SL 75 102 was a weaker agonist than was GABA itself: to evoke equal amplitude responses the concentration of SL 75 102 in the drop usually had to be 10 to 20 times the concentration of GABA. A few cells were very much less sensitive than this to SL 75 102 (e.g. Figure 2), so that the mean relative concentration evoking equal amplitude responses to GABA and to SL 75 102 was 1:33 (range 1:4 to 1:200; $n = 31$).

Voltage-dependence of responses

As a further test of the similarity of responses to SL 75 102 and to GABA, the voltage-dependence of the responses was examined. Membrane voltage displacements were performed in three ways. Firstly steady-state superfusions of high potassium solutions (addition of 6 to 50 meq K^+; osmolarity corrected to within 10 mM) were used to depolarize 10 cells by 6 to 22 mV. On 9 neurones there was a parallel reduction in the amplitude of responses to GABA and SL 75 102.

Secondly 7 cells were depolarized or hyperpolarized by passing d.c. current through the recording micropipette. A careful selection was made for electrodes displaying maximal stability and minimal rectification, but absolute determinations of membrane potential deflections are never possible with this technique. Nonetheless it is valid to compare the changing amplitude of responses to two agonists as long as these are tested close together temporally. With electrodes of resistance 22 to 60 MΩ (mean 34 MΩ), currents of up to about 2 nA were used to displace the membrane potential of the cells (resting potentials 35

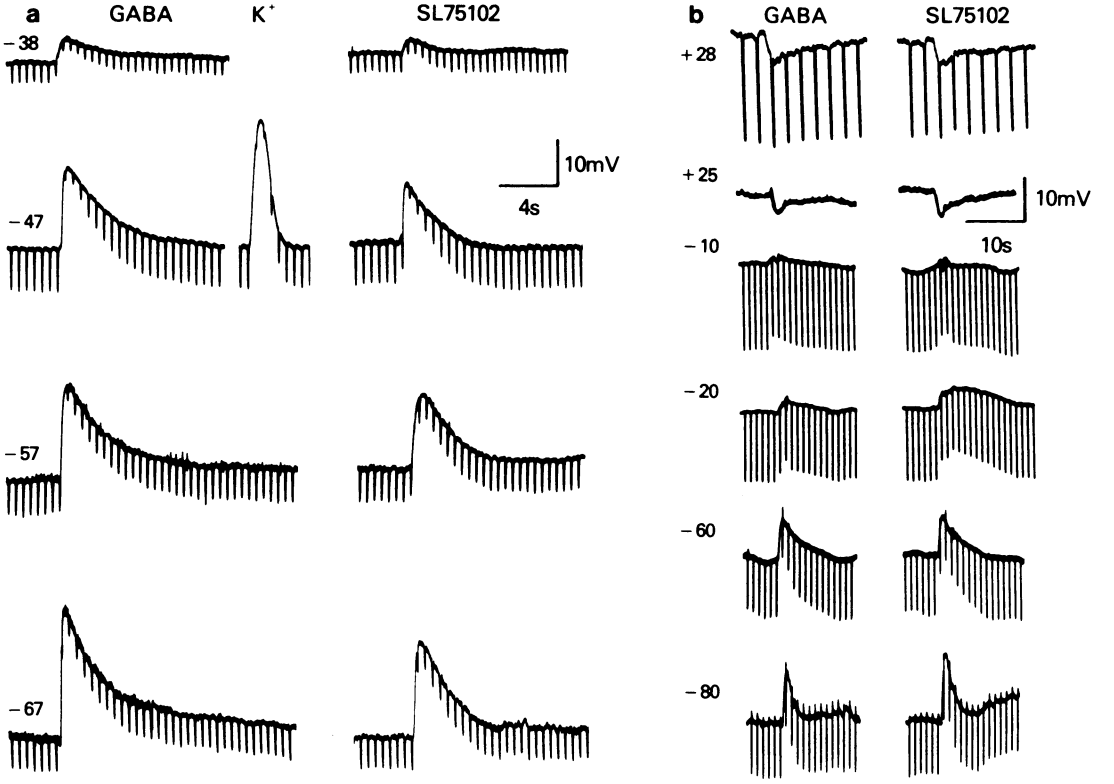

Figure 3 Similarity of voltage-dependence of response to γ-aminobutyric acid (GABA) and SL 75 102. Two intracellular pipettes allowed independent current injection and measurement of membrane potential. In (a) the resting potential was −40 mV and the input resistance 7 MΩ. In (b) the initial potential was −40 mV and the resistance 4 MΩ, but the potential declined during recording to a final value of −20 mV. Hyperpolarizing pulses in (a) were of 1 nA and 60 ms at 1.8 Hz; in (b) they were of 3 nA and 100 ms at 1 Hz. On these cells up to 10 nA of d.c. current was passed for 30 to 60 s to effect the potential changes. All responses were elicited by administering 5 μl drops of the relevant solution into the superfusate. Estimated final concentrations were, in (a) GABA 0.2 mM, SL 75 102 20 mM, KCl 20 mM; in (b) GABA 0.1 mM, SL 75 102 10 mM. All the responses obtained with these cells are plotted on the graphs of Figure 4 as open (a) and closed (b) circles.

to 70 mV, mean 57 mV; resting input resistance 4 to 16 MΩ, mean 10 MΩ). With all 7 cells, the responses to the two agonists were affected in a similar way.

In order to overcome the problems of passing current through the recording pipette, 16 cells were impaled with two independent pipettes. The resting membrane potentials and input resistances of these cells were somewhat lower than for cells impaled with only one electrode. Values with double impalement were 20 to 65 mV, mean 44 mV and 1 to 35 MΩ, mean 7 MΩ.

Figure 3 illustrates the results from two cells, and shows the voltage-dependence of responses to GABA and SL 75 102; it can be seen that the amplitude and timecourse changes of the responses were indistinguishable for the two agonists over a considerable range of membrane potentials. The cell in Figure 3a displayed a marked rectification which can most readily be seen during the transient depolarization elicited by potassium chloride (middle trace on second row). This rectification was a common finding and frequently precluded cells being depolarized as far as the GABA reversal potential. The cell in Figure 3b was one of the few cells which displayed anomalous rectification (i.e. increased input impedance at more positive resting potentials) and which could therefore be depolarized more readily; this depolarization was further facilitated on this cell by a progressive fall of the resting potential during the period of recording. Again, both responses evolved in a parallel fashion and both reversed at about 0 mV.

The results from 9 cells with which the most complete data were collected are presented in graphical form in Figure 4; the amplitude of individual responses is plotted against membrane potential. Although there is considerable scatter between cells, the slope for each cell, and the intercepts on the abscissae (i.e. the reversal potentials) are indistinguishable for GABA and SL 75 102.

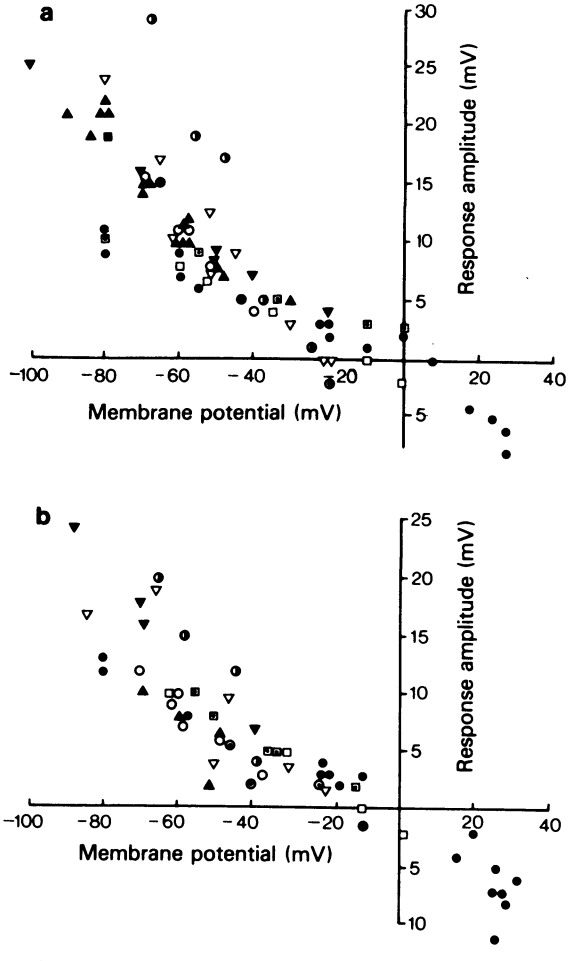

Figure 4 Voltage dependence of responses of 9 cells to γ-aminobutyric acid (GABA) (a) and SL 75 102 (b) expressed in graphical form. Each symbol represents a different cell. All cells were impaled with independent recording and current-passing pipettes. The resting potentials of these cells were 20 to 60, mean 45 mV; resting input resistance 2 to 35, mean 9 MΩ. Depolarization upwards on ordinate.

Chloride-dependence of responses

The above tests indicate that the mechanisms of GABA and SL 75 102 responses are very similar. This was further examined on 4 cells with which agonist responses were elicited regularly before, during and after superfusions of 50% normal chloride solutions (15 to 38 min superfusions; chloride substitution with sulphate and osmolarity correction with sucrose). Although the resting potential is little affected (<2 mV) by such superfusions, responses to GABA decline as a result of a redistribution of chloride consequent on GABA-induced chloride fluxes (see Desarmenien *et al.*, 1980). In the present tests, responses to GABA and SL 75 102 declined (by about 30%) in parallel on 3 of the 4 cells; no effect was seen with the other cell.

Desensitization

Repeated administrations of GABA elicit progressively smaller responses, and this effect can be shown to be due to receptor desensitization (Desarmenien *et al.*, 1979b, 1980). Successive responses to SL 75 102 display a similar pattern, suggesting that this compound acts like GABA to desensitize GABA receptors. This proposition is supported by the cross-densitization that can be shown between SL 75 102 and GABA; Figure 2b illustrates that iontophoretically-evoked GABA responses are reduced to a simi-

lar extent by drop administration of either GABA or SL 75 102. Such administrations of SL 75 102 were seen to reduce iontophoretic GABA responses (elicited 5 to 15 s after the drop of SL 75 102) in 10 of 13 tests, and two-way cross-desensitization was observed in all 7 tests with iontophoretic GABA and in 2 of 4 tests with GABA by drop.

Sensitivity to bicuculline and picrotoxin

Bicuculline (1 to 10 μM, 12 cells) and picrotoxin (1 to 10 μM, 6 cells) reversibly reduced the voltage and conductance responses to both GABA and SL 75 102. On 10 cells the responses to the two agonists were reduced in parallel, but on two occasions the SL 75 102 responses were somewhat less sensitive to bicuculline than were the GABA responses. Figure 5 illustrates the bicuculline sensitivity of responses to both GABA and SL 75 102. In this and in tests on 6 other cells, a cycle was established of drop administrations of GABA and SL 75 102 and of iontophoretic GABA, all at 1 min intervals to avoid problems of desensitization. Into this cycle was usually introduced a response to KCl, which acted as a control response which should be resistant to the GABA antagonists and so monitor the stability of the superfusion and the constancy of the drop administrations. The cycle was repeated at least three times before the superfusion medium was changed to a solution containing one of the antagonists, and thereafter until the antag-

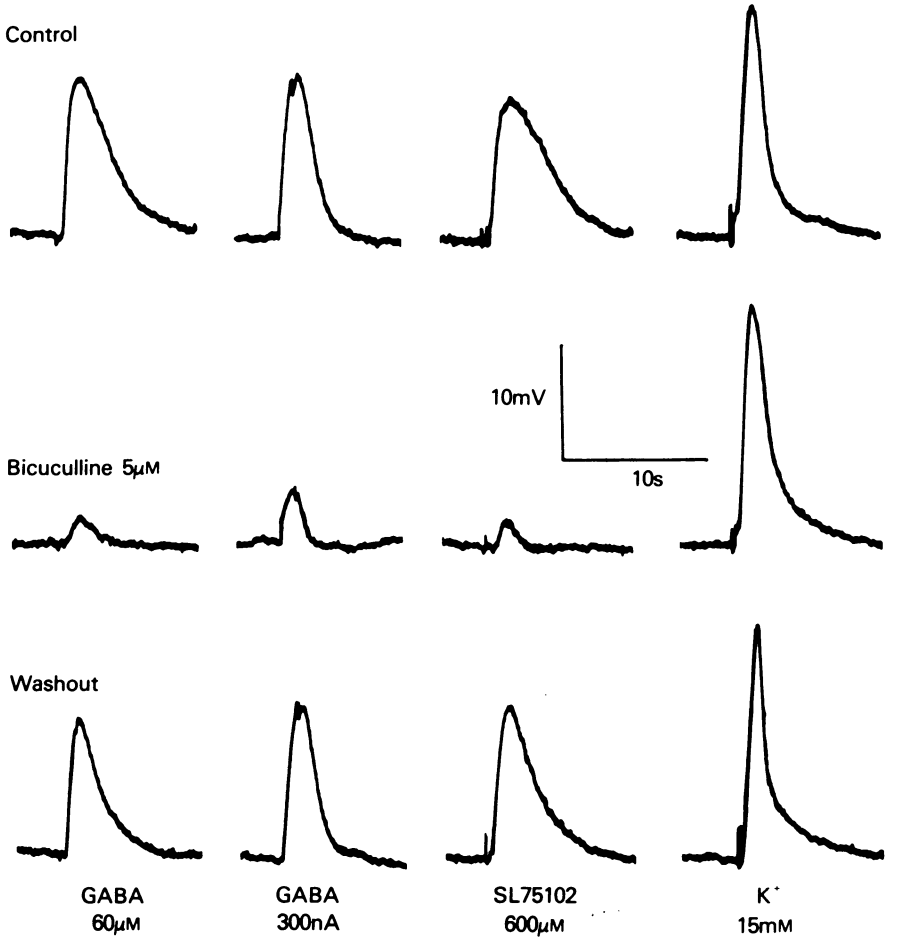

Figure 5 Parallel antagonism by bicuculline of responses of a DRG neurone to γ-aminobutyric acid (GABA) and to SL 75 102. A regular cycle was established consisting of responses to iontophoretic GABA (300 nA, 1 s) and to GABA, SL 75 102 and KCl administered by drop; values indicated are estimated final concentrations. Top row, control. Centre row, 5 min after starting a perfusion of 5 μM bicuculline. Bottom row, 13 min after end of bicuculline perfusion. Cell resting potential −61 mV, resting input resistance 11 MΩ.

onist had achieved a steady-state effect. Normal Ringer was then again superfused until full recovery was observed.

In the example of Figure 5, bicuculline 5 μM reduced the responses to SL 75 102 in parallel with those to GABA. On this cell bicuculline at 1 μM caused a smaller but nonetheless parallel reduction of the agonist responses. Picrotoxin at 1 and 5 μM caused similar partial and almost complete reductions, respectively, of the responses to the same concentrations of the agonists whereas KCl responses remained unaffected in all cases.

Dose-responses curves

SL 75 102 thus displays GABA-mimetic properties but is substantially weaker than GABA itself. It was therefore of interest to try to establish the dose-response characteristics of the depolarizations evoked by the two agonists. Dose-response curves were prepared from 11 cells. During the construction of the curves, GABA and SL 75 102 were usually administered alternately so as to minimize any artefacts due to changes of membrane potential, superfusion flow characteristics or cell sensitivity. The administration sequence was in steps usually of increasing, but occasionally of decreasing, doses. Intervals of 1 min were allowed between administrations. In the example of Figure 6 most point are the means of 2 to 3 responses, but recording conditions were not always sufficiently stable to allow such repetitive administrations to be made. On this cell SL 75 102 was, typically, some 10 times weaker than GABA; unusually, however, the maximum amplitude attained was somewhat lower than the maximal response to GABA. It was a constant finding that the responses to the very high concentrations of SL 75 102 (>1 mM) were shorter in time-course than were the lower concentrations which evoked just submaximal responses; this effect has not been investigated further.

On two occasions recording conditions were sufficiently stable to allow picrotoxin (1 cell) and bicuculline and picrotoxin (1 cell) to be tested for effects on the dose-response curves. The curves to GABA and SL 75 102 were affected in a similar manner, but the cell tested with bicuculline was one of the two cells referred to above on which SL 75 102 was less sensitive to bicuculline than was GABA. The slope of the curves was unaffected by bicuculline and on one of the cells by picrotoxin. In the example of Figure 6, however, picrotoxin 5 μM reduced the slope of the curves to both GABA and SL 75 102, and also reduced the maximal response attained.

On one of these cells an analysis of membrane input resistance was carried out. Figure 7 shows the parallel shift to the right, by bicuculline 1 μM, of the SL 75 102 curve. The rectification of this cell was measured during depolarizations elicited by drop ad-

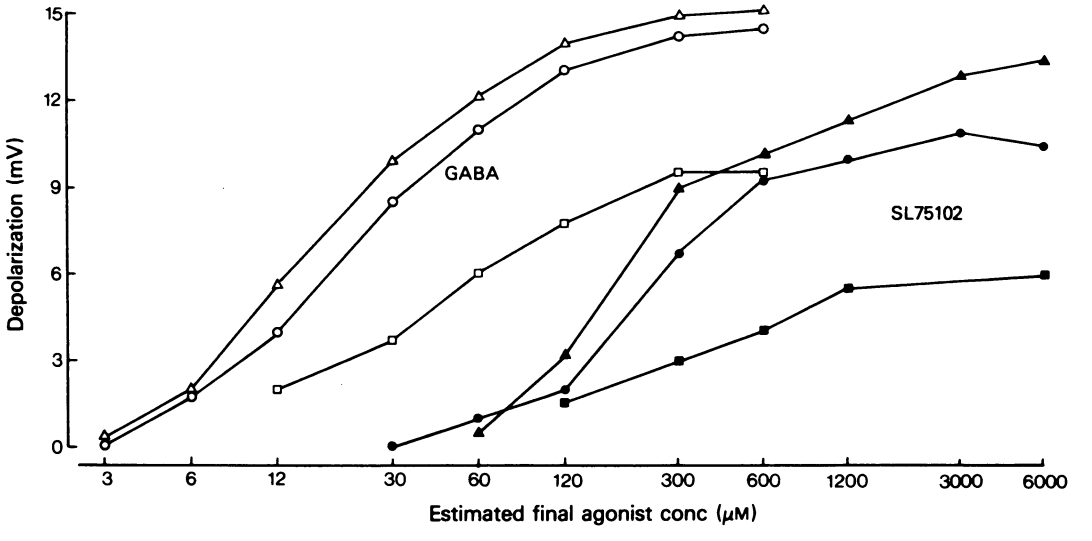

Figure 6 Effects of picrotoxin (5 μM) on log dose-response curves to γ-aminobutyric acid (GABA) and SL 75 102. Responses are depolarizations recorded in a 67 × 55 μm DRG neurone with resting potential of −73 mV. Administration of agonists by drop. Symbols for GABA: (○) control; (□) starting 63 min after start of picrotoxin 1 μM superfusion and 6 min after start of picrotoxin 5 μM superfusion; (△) starting 26 min after end of picrotoxin superfusion. SL 75 102: (●) control; (■) picrotoxin; (▲) washout (timing as for GABA). Most points are means of 2 to 3 responses. Note that the curves for GABA and SL 75 102 are parallel. Picrotoxin caused a shift to the right, a decrease of the slope and a reduction of the maximal response to both agonists.

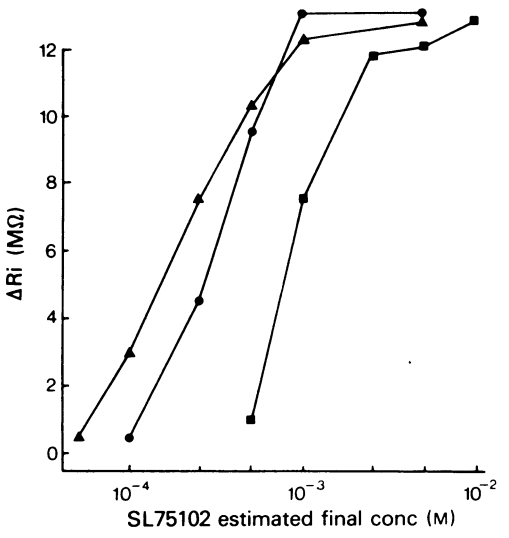

Figure 7 Effects of bicuculline (1 μM) on log dose-response curves to SL 75 102. In this case (cf. Figure 6) the response is the change in membrane input resitance (Δ Ri), measured with constant current hyperpolarizing pulses (1.0 nA, 300 ms, at 1.2 Hz). DRG neurone with resting potential of −76 mV and input resistance of 17 MΩ (latter measured at time of penetration 2 h 15 min previously). Administration of SL 75 102 by drop. The control curve (●) was begun 13 min after the end of a superfusion of picrotoxin 1 μM (full recovery had occurred); bicuculline curve (■) 7 min after start of bicuculline superfusion; washout curve (▲) 7 min after end of bicuculline superfusion. Most points are based on only a single response to SL 75 102.

ministrations of various concentrations of KCl. Extrapolation suggests that the maximum error of membrane input resistance during the largest depolarizing responses to SL 75 102 could be 1 to 2 MΩ.

Discussion

Methods used

The technique of administration of compounds by drop has a number of advantages. Firstly one can achieve a rapidly-rising concentration at the level of the cell, as shown by the rapid leading edge of potassium responses (Figure 5). Secondly the concentration reaching neuronal receptors on any one cell should be more even than is likely with iontophoretic administrations; in particular, very high, very localised concentrations will be avoided (although in fact we tried to reduce this problem in iontophoretic tests by positioning the iontophoretic pipette at some distance from the cell upstream in the flow of Ringer). Thirdly a reasonable estimate of the final concentration reaching the cell under study can be made; a dilution factor can be estimated by comparing the depolarizations evoked by KCl solutions by superfusion and by drop, although such estimates do not take into account any difference in the access to the neuronal membrane between K^+ ions and the other compounds administered. Such differential access could be caused by the satellite glial cell layer, although it is to be noted that glial uptake of GABA does not affect responses of surface DRG neurones to GABA administered either by iontophoresis or by drop (Desarmenien, Feltz & Headley, 1979a; 1980); the apparent discrepancy between this result and the opposite finding in sympathetic ganglia by Brown & Galvan (1977) is discussed in Desarmenien *et al.* (1980) and in Headley, Desarmenien, Linck, Santangelo & Feltz (1980). Fourthly solutions can be tested immediately after being prepared. This was particularly important in these tests because of the instability of SL 75 102; indeed, since we always used solutions less than one hour old, it was often necessary to change the solution of SL 75 102 several times during the recording of one cell; it was thus clearly impractical to test this compound by iontophoresis.

The disadvantage of the technique is that any variation in the placing of drops can result in considerable fluctuations of response amplitude; this would be expected since the drop was not diluted evenly throughout the superfusate. Nonetheless the volume of the initial drop (5 μl), and the degree of dilution, were such as to ensure that the peak concentration attained by any one drop would be even over the exposed surface of the cell under study.

SL 75 102 as a GABA-mimetic agent

The evidence that SL 75 102 is an agonist at GABA receptors can be considered from three points of view. The first and fundamental finding is that SL 75 102 elicits depolarizing responses in DRG neurones; such responses are almost identical with responses to GABA and muscimol in terms of membrane potential and input resistance. SL 75 102 was, however, some 33 times weaker. This value is in reasonable accord with values obtained in tests of displacement of the binding of [^{3}H]-GABA to membrane fractions of rat brain: in these tests the IC_{50} of SL 75 102 (the concentration displacing 50% of bound [^{3}H]-GABA) was some 23 times higher than the IC_{50} of GABA itself (Bartholini *et al.*, 1979; Kaplan, Raizon, Desarmenien, Feltz, Headley, Worms, Lloyd & Bartholini, 1980).

Secondly it seems that both agonists cause this depolarization by a similar ionic mechanism since the voltage-dependency of the responses was the same; in addition the responses to both compounds were affected similarly by alterations of the chloride concen-

tration of the superfusate. These results are therefore consistent with a primary action of SL 75 102 on chloride channels, as is the case with GABA (see Introduction for references).

Thirdly, evidence that the same receptors are activated by the two compounds stems from experiments on desensitization and with GABA antagonists. The two-way cross-desensitization displayed is most readily explained by an action at a common receptor (we have previously shown that desensitization at this site is not related to GABA uptake processes; see Desarmenien *et al.*, 1979a, 1980). The GABA antagonists, bicuculline and picrotoxin, reversibly reduced responses to SL 75 102 and GABA to a similar degree on 10 of 12 cells examined; on 2 cells, however, SL 75 102 was relatively resistant to bicuculline, but our data do not allow further analysis of this finding.

The log dose-response curves plotted for depolarizations by GABA and SL 75 102 were reasonably parallel and the antagonists bicuculline and picrotoxin affected the curves in a similar fashion. The reduction of slope and of maximal responses seen on one cell with picrotoxin should be treated with caution since the curves are plotted on the basis of membrane voltage, rather than current, responses: they are therefore prone to errors introduced by voltage-dependent conductance changes. That this was not, however, an important factor is suggested by the finding that conductance changes during responses to KCl were only a small proportion of the conductance changes during similar-sized GABA responses, especially with responses of up to 10 mV amplitude.

The interpretation of the results with the antagonists depends on whether the antagonism is competitive or non-competitive. Picrotoxin is generally considered to act at the level of chloride channels (see review by Nistri & Constanti, 1979). Bicuculline, on the other hand, is a competitive antagonist in several tissues, although Gallagher *et al.* (1978) found it to be non-competitive in DRG of cats. Our only evidence on rat DRG was the finding with one cell that bicuculline caused a parallel shift of the GABA dose-response curve.

Stability of SL 75 102

As stated above, SL 75 102 dissociates slowly under *in vitro* conditions to release free GABA. In the absence of biological tissue this dissociation proceeds at less than 5% per hour, but if the Schiff link dissociates more rapidly when in contact with biological material, then our results could be explained by activity of released GABA. For this to be the case it would be necessary for 1 to 25% of the SL 75 102 to dissociate within 1 to 2 s of contact with the DRG. This seems unlikely since SL 75 102 displaced [^{3}H]-GABA from membranes prepared from human or animal brains under conditions (5 min, 0°C) where little if any dissociation of the Schiff bases occurs (K. G. Lloyd, unpublished results); its affinity in these tests was one third to one twentieth that of GABA itself (Bartholini *et al.*, 1979).

Conclusion

The evidence presented above is consistent with an action by SL 75 102 on GABA receptors, and it seems likely that under *in vitro* conditions this is a direct effect by the undissociated compound. However, it remains to be established what the relative roles are *in vivo* of the parent compounds SL 75 102 and SL 76 002, and of their dissociation products, GABA and GABAmide respectively.

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