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DOES GLIAL UPTAKE AFFECT GABA RESPONSES? AN INTRACELLULAR STUDY ON RAT DORSAL ROOT GANGLION NEURONES *IN VITRO*

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SUMMARY

1. Using single barrel pipettes, intracellular records were obtained from surface neurones of isolated rat dorsal root ganglia (DRG) impaled under microscopic vision.

2. Responses to γ-aminobutyric acid (GABA) were elicited either by ionophoresis or by placing drops of concentrated GABA solutions directly into the flow of superfusing Ringer. Using this latter method it was estimated that the GABA concentration eliciting threshold ($\simeq$ 1 mV) responses was 3–20 μM.

3. Short ($\leqslant$ 1 sec) ionophoretic or drop administrations of GABA elicited depolarizing responses associated with an increased membrane conductance. With longer applications the initial depolarization was not sustained but decayed to a lower plateau level (desensitization) associated with a minimal conductance change.

4. Low chloride superfusions did not affect subsequent responses to GABA unless GABA was also administered during the low chloride superfusion, in which case responses declined markedly. This suggests that GABA caused appreciable chloride fluxes when it was administered regularly (e.g. for 1 sec every minute).

5. Glial GABA uptake was inhibited by adding 1 mM-β-alanine or 0·25 mM-chlorpromazine to the bicarbonate-Ringer superfusate or by substituting lithium for sodium in a Tris-Ringer superfusate. Uptake inhibition had no consistent effect on any of the parameters studied, namely membrane potential, input resistance, amplitude and time course of responses to GABA, and GABA desensitization.

6. Muscimol and isoguvacine, which are probably not substrates for the glial GABA carrier, elicited responses with time course and desensitization characteristics indistinguishable from those of responses to GABA.

7. GABA superfused at concentrations as low as 1 μM could reduce responses to ionophoretic GABA, i.e. cause a desensitization of GABA receptors.

8. It is concluded firstly that in DRG, glial uptake does not affect the amplitude or time course of responses to GABA when the neurone under study is close to the source of GABA; and secondly that desensitization can occur independently of GABA uptake.

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9. The findings are discussed in relation to their possible relevance to GABA systems in the central nervous system.

INTRODUCTION

γ-aminobutyric acid (GABA) is well recognised as an inhibitory neurotransmitter in the mammalian central nervous system. There is a wealth of information available on the cellular and membrane actions of this amino acid (reviews by Curtis & Johnston, 1974; Kelly & Beart, 1975; and see Krogsgaard-Larsen, Scheel-Krüger & Kofod, 1979, for a recent symposium proceedings) but certain aspects of the actions of GABA remain ill defined. In the investigation reported here we have examined two such aspects, namely the role of glial uptake of GABA in determining the characteristics of neuronal responses to GABA, and also the process of desensitization of GABA receptors; and we comment on the possible relation between these two processes.

In the mammalian central nervous system, micro-electrophoretically administered GABA normally evokes monophasic plateau inhibitory responses; desensitization is not apparent. On the other hand some mammalian studies have shown that the actions of GABA can be prone to a desensitization process (Curtis, Phillis & Watkins, 1959; Krnjević & Phillis, 1963; Dreifuss, Kelly & Krnjević, 1969; Krnjević, Puil & Werman, 1977; other authors have described similar phenomena in non-mammalian preparations, for instance Diamond & Roper, 1973). The possible physiological significance of such desensitization is unclear; moreover the underlying mechanism has not been ascertained. To date only one study in mammals has been directed specifically towards this question: Krnjević *et al.* (1977) considered two main alternative causes (1) true desensitization ('slow conversion of receptors to an inactive form') and (2) apparent or false desensitization resulting from changing extracellular GABA levels caused in turn by a GABA-induced stimulation of neuronal uptake of GABA; these authors concluded that 'desensitization' was most likely to be related to uptake processes.

GABA uptake has been postulated to be an important factor in terminating the action of GABA (see review by Iversen & Kelly, 1975). In electrophoretic tests in the central nervous system, inhibitors of GABA uptake do indeed potentiate the depressant action of GABA although they have no effect on synaptic transmissions thought to be mediated by GABA (Curtis, Game & Lodge, 1976; Lodge, Johnston, Curtis & Brand, 1977; see also Lodge, Curtis & Johnston, 1978). In the central nervous system, GABA is known to be taken up into both neuronal (principally nerve terminal) and glial elements (see Iversen & Kelly, 1975), but the relative importance of these two systems in affecting the characteristics of GABA responses has not yet been clarified.

We have investigated some of these aspects of responses to GABA by studying neurones in dorsal root ganglia (DRG). In practical terms the use of the DRG offers several advantages: (1) the structure has been well described (see review by Lieberman, 1976); (2) there is a GABA uptake system and at low substrate concentrations this is limited to the satellite glial cells (Schon & Kelly, 1974*a*); (3) there are no synapses in this ganglion (unlike other peripheral ganglia) and so neuronal responses

can be examined in the absence of indirect synaptic effects; and (4) the ganglia can be superfused with minimal diffusion barriers between cell and superfusion medium, particularly in *in vitro* preparations which allow recordings to be made from cells on the surface of the ganglion. A fundamental aspect of this preparation is that the somata of DRG neurones possess GABA receptors; moreover the characteristics of the responses of such neurones to GABA have already been described (de Groat, Lalley & Saum, 1972, rat *in vivo*; Feltz & Rasminsky, 1974, rat *in vivo*; Nishi, Minota & Karczmar, 1974, frog *in vitro*; Deschenes, Feltz & Lamour, 1976; Deschenes & Feltz, 1976, rat *in vivo*; Gallagher, Higashi & Nishi, 1978, cat *in vitro*; see also the studies on cultured DRG neurones by Obata, 1974; Lawson, Biscoe & Headley, 1976; Hösli, Andrès & Hösli, 1977; Ransom, Bullock & Nelson, 1977). Thus short administrations of GABA to DRG neurones result in depolarizing responses mediated principally if not exclusively by an increase in chloride conductance. These responses are apparently similar to those occurring at the intraspinal terminals of the same cells, these being the terminals implicated in spinal presynaptic inhibition (for reviews see Curtis, 1978; Levy, 1978). Longer GABA administrations, however, result in biphasic responses: the initial voltage and conductance change decays to a lower level (desensitization) which is maintained for the duration of the administration. These various properties are similar to those of sympathetic ganglion neurones, for which there is evidence that glial uptake can influence neuronal responses to GABA (Bowery, Brown, Collins, Galvan, Marsh & Yamini, 1976; Brown & Galvan, 1977); this process has not, however, been investigated at the cellular level.

We report below that altering the rate of GABA uptake into satellite glial cells affects neither the amplitude nor the time course of responses of DRG neurones to this amino acid, and that desensitization is unchanged during uptake inhibition. Some of the results have been communicated in preliminary form (Desarmenien, Feltz & Headley, 1979*a*, *b*).

METHODS

Previous reports from this laboratory have concerned experiments on rat DRG *in vivo*. Because of the restricted visibility in these experiments, recordings could not reliably be made from cells on the surface of the ganglion; it is, however, advantageous to record from such surface cells firstly to limit the diffusion barriers between cells and the superfusion medium and secondly because independent positioning of micro-electrophoretic pipettes then becomes feasible. In the present study we have therefore used an *in vitro* preparation.

Rats were killed by cervical dislocation after being stunned, and the spinal column was excised rapidly, hemisected and placed in a modified Krebs solution (for composition see below) at room temperature. Lumbar ganglia were then dissected out together with several millimetres of spinal roots and peripheral nerve trunk. The largest ganglia in the lumbar enlargement were normally used since these might be expected to contain the highest proportion of big ($> 40\ \mu m$ diameter) neurones (see Lieberman, 1976). The nerve ends were tied and the ganglia pinned on a layer of transparent silicone rubber (RTV 141, Rhône-Poulenc) in a chamber which used a microscope slide as its base. The connective tissue sheath on the upper surface of the ganglion was then removed with forceps. It was our impression that this could be achieved more satisfactorily, and without enzyme treatment, when young rats were used. Most experiments were therefore performed on rats weighing 60–120 g (3–5 weeks old). DRG neurones of such animals should, however, have reached nearly mature size (see Lawson, Caddy & Biscoe, 1974).

The chamber was then placed on the stage of a modified Leitz Ortholux II microscope and superfused with the Krebs solution at 30–35 °C. Using a long focal distance objective and final magnification × 250– × 320, single cells could readily be distinguished.

Single barrel intracellular micropipettes were filled with 0·5 M-potassium sulphate or with 4 M-potassium acetate (initial resistance 20–80 MΩ). Electrodes were advanced using a stepping motor manipulator but final penetration of surface cells was usually achieved by vibrating the tip of the electrode by causing the 'negative capacitance' circuit to oscillate.

The greatest problem in maintaining stable intracellular records was in keeping a constant level of superfusate above the ganglion. This level had to be as low as possible to optimize visibility, but in consequence any small fluctuations in the level altered the surface tension exerted on the fine terminal part of the pipette, which was necessarily angled at about 45°, and so caused the pipette to move; the result was often a small shift in the DC level of the signal being recorded. For this reason it is difficult to interpret the small and slow changes in apparent membrane potential seen on occasion during superfusion of the solutions used. An additional problem was that changing fluid levels could have affected the diffusion of GABA from the ionophoretic pipette to the cell. Some of these problems might have been attenuated by using a water-immersion lens but the more horizontal electrode approach necessitated would have caused yet other problems.

The composition of the Ringer solution, which was equilibrated with 95% O_2: 5% CO_2, was: NaCl 124 mM; KCl 2 mM; KH_2PO_4 1·25 mM; $MgSO_4$ 2 mM; $CaCl_2$ 2 mM; $NaHCO_3$ 25 mM and glucose 11 mM. Low chloride solutions were prepared by replacing NaCl with Na_2SO_4, osmolarity correction being made with sucrose; the other constituents remained the same. In experiments in which sodium-free solutions were used, a Tris-Ringer was prepared, of composition: Tris base 8 mM; Tris HCl 42 mM; either NaCl or LiCl 99 mM; KCl 2 mM; KH_2PO_4 1·25 mM; $MgSO_4$ 2 mM; $CaCl_2$ 2 mM and glucose 11 mM. Changing from bicarbonate to Tris-Na^+-Ringer did not affect neurones in any consistent way.

In most experiments GABA was administered by microionophoresis. Ionophoretic pipettes contained GABA 1 M, or, in some experiments, muscimol 100 mM or β-alanine 1 M, all at pH 3–4. Because of the rapid desensitization of GABA responses, it was important to study responses with a relatively fast time course; this necessitated positioning the ionophoretic pipette within about 50 μm of the cell under conditions of minimal tissue barriers (i.e. surface cells and little or no surface connective tissue; see also below). Nonetheless direct contact of the pipette with the ganglion, and in particular with the cell under study, was avoided so as to prevent current-induced artifacts; this procedure did, however, necessitate the use of larger ejecting currents. Similarly, to avoid current artefacts on the silver-silver chloride indifferent electrode, a separate pipette was used for the ionophoresis ground return path.

Whilst ionophoretic administrations evoke controlled and reproduceable responses, it is not possible to determine the concentration attained at the level of the cell being recorded. In principle the simplest way to overcome this problem would be to add GABA in known concentrations to the superfusion medium. In practice it is not feasible to attain equilibrium concentrations within the 1–5 sec before the onset of desensitization. We have therefore devised an indirect test as follows: short-lasting responses with a rapidly rising leading edge can be evoked by placing a drop of solution ($\leqslant 5\,\mu$l. from a Hamilton syringe) in the chamber between the entry of the superfusion medium and the ganglion. By comparing the depolarization evoked by a drop of potassium and by superfusions of known concentrations of potassium, a 'dilution factor' could be estimated for each cell for such administrations by drop. We have made the assumption that the same dilution factor applies to drop administrations not only of potassium ions but also of the other compounds so tested. This assumption seems reasonable in view of the minimal tissue barriers between the superfusion medium and the surface cells studied.

RESULTS

The results described here were obtained from 116 cells in lumbar ganglia taken from sixty-nine rats.

Properties of the neurones recorded. Stable resting potentials (V_m) were maintained for up to 12 hr per cell; many of the results described below were obtained from cells with V_m changes of no more than 5 mV per hour. After initial penetration, and before commencing pharmacological tests, a hyperpolarizing current of 0·05–0·5 nA

was often passed for 5–10 min to help stabilize the membrane potential; the V_m often increased by 5–20 mV over this period. Very occasionally such a hyperpolarizing current was maintained during tests.

Records were taken from cells with V_m between 35 and 90 mV; ninety-seven of the 116 cells had V_m between 50 and 75 mV.

The apparent input impedance (R_m) of these cells, measured using a single barrel pipette, varied from less than 1 to over 100 MΩ with 68% of values lying between 4 and 20 MΩ.

Ninety-nine cells were tested with intracellular current pulses; sixty-seven cells responded with full-sized action potentials, such as that illustrated in Fig. 1*C*; 'inactivated' ($\leqslant$ 5 mV) action potentials were seen with seventeen cells and no active response could be evoked with the remaining fifteen cells. Because of the difficulty of adjusting the 'capacitance neutralization' circuit, particularly when high impedance electrodes were used, it is not possible to specify accurately the height of action potentials. With the settings used spikes normally reversed within ± 10 mV of zero (see Fig. 1*C*). Spikes usually had fast rising and falling phases, but occasionally longer-lasting action potentials were seen. Such long duration spikes are often calcium-mediated (see Yoshida, Matsuda & Samejima, 1978, and references therein) but this property has not been investigated in the present study.

Characteristics of responses to GABA. Short ($\leqslant$ 1 sec) ionophoretic administrations of GABA elicit depolarizing responses in rat DRG neurones *in vitro* similar to those described in other DRG preparations including rat *in vivo* (see Introduction). The responses to such short ejections of GABA increase in amplitude with increasing ionophoretic currents until a peak depolarization is reached. Under our conditions maximal depolarizations of 15–30 mV, depending on the cell, were obtained with ejection currents of 50–400 nA. Longer ionophoretic administrations of GABA, however, evoke more complex responses which have not previously been fully described. With maintained currents which are subthreshold with ejection periods of $\leqslant$ 1 sec, there is a slow depolarization which reaches a steady plateau of only a few mV after some 10–30 sec. We have seen such responses with currents as low as 1 nA. Very small increases in current increase both the rate of rise of the depolarization and the amplitude of the plateau, as would be expected from an increased release from a point source. Larger currents increase the rate of rise and the amplitude of the early part of the response, but this depolarization is not maintained: it falls to a lower plateau level, thereby giving a biphasic response pattern, and indicating that some sort of desensitization process occurs. Further current increases evoke progreessively larger first phases but fail to alter either the amplitude of the second phase of depolarization or the membrane resistance during the second phase. The higher the ejecting current, the larger the first phase and the faster the decline to the second phase plateau, but because of limitations of the ionophoretic technique, the precise dose–response relationship cannot be determined. Typical biphasic responses can be seen in Fig. 1*A* and *B* in response to GABA administrations lasting respectively 15 and 11 sec, and in Fig. 6 in response to applications of 37–40 sec.

The simplest explanation of the second phase of the biphasic responses is that there is a sustained small increase in chloride conductance and that this causes the maintained small depolarization. The signal:noise ratio of most of our records

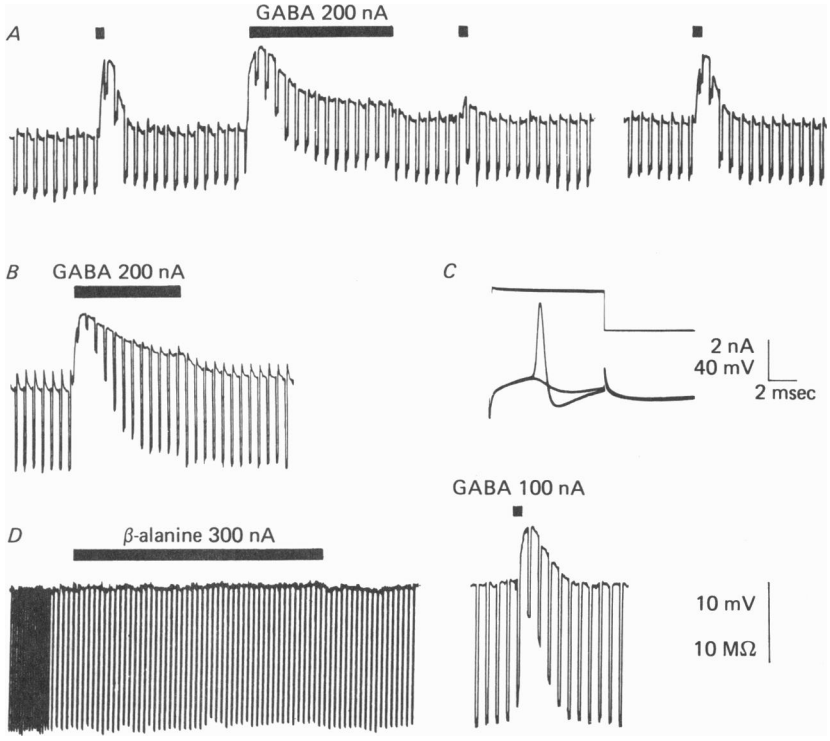

Fig. 1. Intracellular records of neurones in rat dorsal root ganglia *in vitro*. Depolarization is upwards in this and all succeeding figures. The hyperpolarizing current pulses in *A*, *B* and *D* were of 1 nA and 300 msec and provide a measure of resting membrane resistance. *A*, the black bars above the trace indicate the ionophoretic administrations of GABA from a neighbouring pipette. Note that the first short ejection (1 sec) provoked a depolarization associated with an increased conductance. The ensuing 15 sec ejection elicited a biphasic response: the first phase decayed (desensitization) to a lower amplitude depolarization which was associated with a minimal conductance change. A short ejection 8 sec after the end of the previous longer ejection evoked only a very small response, but the final ejection (2 min later, after the break in the record) evoked a response comparable with the initial control response. Neurone with resting potential of -71 mV; resting input resistance of 7 MΩ. *B*, in this cell the second phase of depolarization was associated with a small decrease in conductance, suggesting that the mechanism of this second phase of depolarization is different to that of the first phase. Resting potential -68 mV; input resistance 10 MΩ. *C*, example of an intracellularly evoked action potential. The top trace shows the 2 nA, 8 msec depolarizing current pulse which was used to trigger the oscilloscope. Most cells, like this one, displayed brief action potentials. Resting potential -60 mV. *D*, β-alanine, which inhibits DRG glial GABA uptake, had no effect on either resting potential or resistance when ejected with currents of up to 300 nA. In contrast GABA (100 nA, 1 sec in this case) provoked the usual depolarizing response. Break in record is 2·5 min. Resting potential -60 mV; input resistance 11 MΩ. (The bridge system was not in balance at this point due to an increase in electrode resistance, as verified when the electrode was withdrawn from the cell). Calibration: in *A*, *B* and *D* the voltage calibration is that given beside *D* and the time course is given by the hyper-polarizing pulses which were at 1 Hz. The calibration beside *C* applies to *C* only.

precluded an accurate comparison of the ratio of voltage to conductance change during the two phases of the response, principally because the amplitude of the second phase was usually too small to be associated with an easily measurable conductance change. Nonetheless records such as that of Fig. 1 *B* suggest that some other process is responsible for the second phase of depolarization, for in this case the conductance increase during the first phase was particularly large, yet no conductance increase was detectable during the second phase, the amplitude of which was 25 % of that of the first phase; indeed careful scrutiny suggests a small *decrease* in conductance during the plateau of the second phase.

One common finding in our experiments was that the amplitude of responses to GABA declined slowly during the period of recording from any one cell (e.g. Fig. 2). It was important for the interpretation of the pharmacological tests described below to establish the cause of this decline. That it was not due to a deterioration of the preparation was indicated firstly by the continuing stability of the resting potential, and secondly by the fact that other cells with normal responses could be found afterwards. The reduced responses to GABA are thus more readily explained by (i) change in micropipette to neurone distance, (ii) increased diffusional barriers between pipette and neurone, (iii) progressive desensitization or (iv) reduced GABA driving potential due to a loss of internal chloride. The first explanation is unlikely in view of the visual supervision possible. Increased diffusional barriers undoubtedly occured: a layer looking rather like fibrin deposits do in *in vivo* experiments could sometimes be seen to accumulate during recording periods (subsequent histology on three occasions showed this layer to be composed of overgrowing glial and, or fibroblast cells). Nonetheless we found that a reduction of the GABA-mediated depolarization could occur without any change in the GABA-induced conductance increase. This finding precludes an explanation in terms of progressive desensitization. The most likely explanation is thus that the GABA-induced increase in chloride conductance reduced the internal chloride concentration, and hence E_{Cl} became more negative.

To examine the possibility that internal chloride readjusted moderately quickly under our conditions (cf. Gallagher *et al.* 1978) we performed experiments in which the chloride content of the superfusing Ringer was reduced from 130–6 m-equiv. In one series of tests, GABA responses were elicited ionophoretically at regular intervals of 45–90 sec throughout. When the superfusate was changed from normal to low chloride, an initial augmentation of the GABA responses was seen with four of ten neurones. With all ten cells, however, responses to GABA were reduced by 50–100 % after 10–20 min low chloride perfusion, and in two cases GABA transiently caused a small hyperpolarization when normal chloride was again superfused. Full recovery was not seen. On the other hand when GABA was not administered during the low chloride superfusions (15–22 min, twenty cells) then the responses to GABA applied immediately after the replacement of 130 m-equiv chloride were normal in tests on nineteen of the twenty cells. Similarly with two cells responses to GABA were normal when evoked just after the end of superfusions of 52 m-equiv chloride. It therefore seems that the resting chloride conductance is low but that appreciable chloride fluxes take place when GABA is administered repeatedly.

Concentration of GABA attained during the responses. In order to be able to relate

GABA response characteristics to uptake processes (see below) it is important to compare the concentrations attained during responses with the kinetic properties of the glial uptake system. With ionophoretic administration one cannot define the concentration reaching the cell. In order to estimate the concentration of GABA attained during responses, we therefore administered GABA by drop directly into the superfusion medium, as described in Methods. Having established a dilution factor for each cell, the concentration of GABA giving threshold ($\simeq$1 mV) or larger responses could be estimated by testing different concentration GABA solutions by drop. Results with more than thirty neurones indicate that the minimum effective concentration of GABA lay, for different cells, between 3 and 20 μM (mean 9 μM).

Specificity of glial uptake inhibitors used. Biochemical experiments by Schon & Kelly (1974*b*) have shown that the high affinity uptake of [^{3}H]GABA by DRG glial cells (apparent Michaelis constant, K_m, of 10 μM) can be inhibited in various ways: thus solutions containing 1 mM-β-alanine inhibit uptake, in an apparently competitive manner, by 62%; 0·22 mM-chlorpromazine inhibits uptake by 50% in a presumably non-competitive manner; and the uptake is sodium dependent so that solutions containing no sodium inhibit uptake by 96%, following a 15 min preincubation in the inhibiting medium. Before testing these procedures on GABA responses it was important to know whether they have direct actions on neurones, although for reasons explained in Methods it is difficult to interpret small voltage shifts following changes of the superfusion medium.

In our experiments neither neuronal V_m nor R_m were affected clearly or consistently by changing the superfusion medium to solutions containing either 1 mM-β-alanine (both parameters tested on eight cells) or 0·25 mM-chlorpromazine (five cells). Changing from bicarbonate-Ringer to Tris-sodium to Tris-lithium Ringer occasionally caused bizarre depolarizations which we were unable to explain.

β-alanine was tested at a tenfold higher concentration (i.e. 10 mM) on seven neurones. A small depolarization ($\leqslant$5 mV) was seen with three cells and an increase in conductance (up to 35%) with two of the four cells tested. With six of the cells GABA responses were either severely reduced or fully blocked during β-alanine superfusion. At this concentration, therefore, β-alanine clearly affected neurones directly.

β-alanine administered ionophoretically with currents of 100–300 nA had no effect on the V_m or R_m of the six neurones tested. GABA was shown to be effective on all these cells (see Fig. 1 *D*).

Does inhibition of glial uptake affect the amplitude of GABA responses? Because uptake inhibitors would be expected to have more obvious effects when the doses of GABA used do not saturate the glial carrier for GABA (see also Bowery *et al.* 1976) we tested the effects of uptake inhibition on small ($\leqslant$3 mV) as well as on larger amplitude responses. Because of the period of desensitization which follows each GABA application, reproduceable responses could rarely be evoked at less than one minute intervals. A prolonged period of stable intracellular recording was therefore necessary to obtain responses to each of several GABA doses before, during and after superfusion with the uptake inhibiting media.

Results were obtained with twenty-one cells on the effects on responses to ionophoretic GABA of superfusions with 1 mM-β-alanine (durations 6–50 min, mean 18

min). Of these cells, seven satisfied the criteria of at least two responses to at least three GABA doses during control, test and recovery periods. In no case was there any enhancement of the amplitude of responses to GABA, but with four cells there was a slight reduction of the larger amplitude responses during β-alanine superfusion. An example of one record obtained is shown in Fig. 2, which is a graph, against time, of the amplitude of responses to four ionophoretic currents of GABA. As can be seen, there was a slow and progressive reduction of the size of GABA responses throughout the 52 min of recording displayed, but there was no increase at all during the 17 min superfusion of β-alanine.

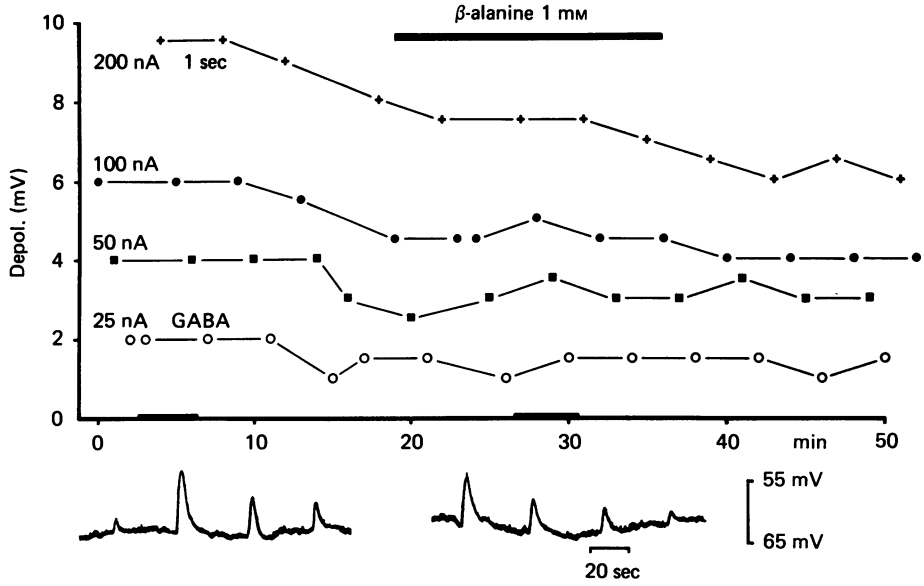

Fig. 2. Graph, against time, of the amplitude of depolarizing responses of a DRG neurone to four ionophoretic currents of GABA before, during and after superfusion of 1 mM-β-alanine. The 1 sec ejections of GABA were at 1 min intervals. The sample records shown at the bottom correspond to the thickened parts of the horizontal base line. Note that the penrecorder was slowed between each response: the time calibration applies to the responses themselves. The amplitude of the responses to GABA, particularly of those to the highest ejection currents, fell progressively during the 52 min of recording shown. Note that there was no enhancement of responses during the 17 min superfusion of β-alanine. The resting potential remained within 2 mV of −64 mV throughout. Input resistance 16 MΩ.

With four cells, 1 mM-β-alanine was tested for effects on responses evoked by drop administration of GABA. In these cases the GABA concentrations eliciting threshold depolarizations were between 5 and 8 μM. No potentiation of responses to GABA was seen during superfusion of the uptake blocker; instead β-alanine caused a 30–60% reduction of the responses of three of the cells to GABA, as in the example of Fig. 3.

β-alanine was also tested by ionophoresis for effects on responses to ionophoretic GABA; five neurones were tested with currents of 100–300 nA β-alanine, for periods of up to 4 min. Again, no augmentation was seen of responses to GABA, whereas a small reduction in response amplitude occurred on three occasions.

High affinity uptake of GABA into DRG is entirely sodium dependent (Schon &

Kelly, 1974*b*). Lithium does not substitute for sodium in glial GABA transport (Brown & Galvan, 1977) but might be expected to substitute for sodium in any small change in sodium conductance which may occur during responses to GABA, although, as stated above, the principal conductance change mediating GABA depolarizations is an increase in chloride conductance. Substitution of sodium by lithium should therefore have caused a reasonably specific but also potent inhibition of GABA transport. Responses to ionophoretic GABA were tested with sodium-free (Tris-lithium) superfusions (duration 11–22 min, mean 15 min) on eight cells and in 25 mM-sodium on one further cell; of these, four cells satisfied the criteria of multiple administrations indicated above. There was no increase of responses to GABA during

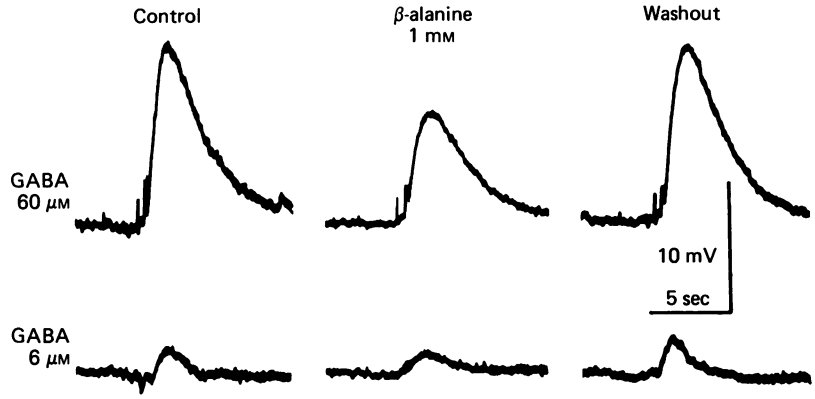

Fig. 3. Responses of a DRG neurone to GABA administered as a drop direct into the superfusion medium. See Methods for description of estimate of final GABA concentration. A 20 min superfusion of β-alanine 1 mM failed to potentiate responses to either GABA 6 or 60 μM; instead the responses were reduced. Cell with resting potential of −62 mV; resting input resistance 11 MΩ.

superfusions of the uptake inhibiting medium. An example is given in Fig. 4: as in the case of Fig. 2, there was a slight reduction of responses during the 40 min recording period shown, and no increase of responses during the 16 min superfusion with zero sodium; indeed the responses to the highest current of GABA were reduced somewhat. On three cells the sodium-free superfusions were tested on responses elicited by GABA administered by drop (threshold responses evoked by 3–5 μM GABA). Again, no potentiation was seen but with one cell the sodium-free solution reduced larger amplitude responses to GABA administered either by drop or by ionophoresis.

Chlorpromazine was tested by superfusion (duration 3–29, mean 12 min) on the responses of eleven neurones to ionophoretic GABA. As with β-alanine and zero sodium, the size of the responses was not affected in most cases, nor was the GABA-induced conductance increase affected in the three cells so tested. With two cells, however, chlorpromazine did cause a small increase of responses to GABA. In one case only the smallest (near threshold) responses were affected, but in the other, responses to all four current levels of GABA were increased in parallel. On two of three cells, chlorpromazine reduced the amplitude of responses elicited by drop (threshold concentrations 6–8 μM); the third cell was not affected.

Responses to muscimol and isoguvacine. Muscimol is a potent agonist at post-synaptic GABA receptors in the central nervous system (Curtis, Duggan, Felix & Johnston, 1971) but is considered not to be taken up by the glial GABA carrier (Johnston, Kennedy & Lodge, 1978). Comparison of GABA and muscimol response profiles can thus lend additional information firstly on the nature of DRG receptors for GABA and secondly on the normal role of uptake in determining the characteristics of responses to GABA.

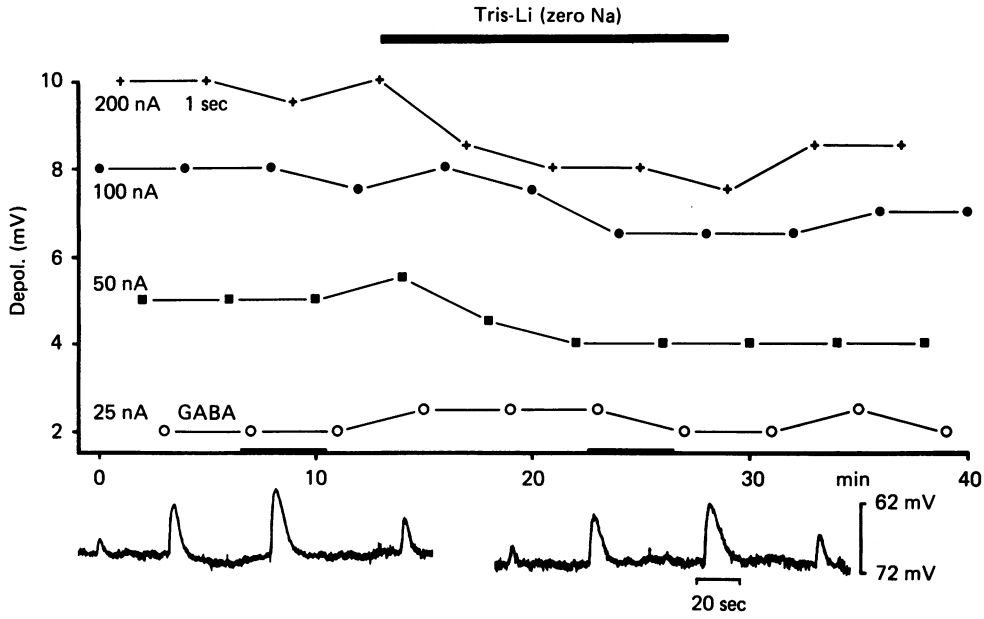

Fig. 4. Effect of a 16 min superfusion of a sodium-free solution on responses of a DRG neurone to four inophoretic currents of GABA. Layout as for Fig. 2. The sodium-free solution was obtained by lithium substitution in a Tris-Ringer. It did not significantly enhance the responses to GABA; the apparent small increase of the 25 nA responses is within the noise level of the records (see sample traces at bottom). Instead the response to the highest current of GABA was reduced somewhat. The resting potential of the cell remained within 2 mV of −71 mV. Input resistance 8 MΩ.

As in previous experiments on rat DRG *in vivo* (Feltz, Deschenes & Desarmenien, 1978; Desarmenien *et al.* 1979*b*), ionophoretic muscimol elicited GABA-like depolarizing responses in all fifteen cells tested. Repeated short administrations of GABA result in progressively smaller responses—one manifestation of the desensitization process. Repeated muscimol ejections gave the same pattern; moreover the same effect was seen in cross-desensitization tests when GABA and muscimol were ejected shortly after each other, in either order (Fig. 5). Similar results were seen on the five cells tested this way, and also on four cells tested with muscimol and GABA by drop. Ionophoretic muscimol was found not to be significantly more potent than GABA, but this is likely to be due to muscimol having a lower transport number than GABA, since muscimol by drop was 2–10 times as potent as GABA (mean 3·7 times, twelve cells). A further finding was that the time course of responses to muscimol was similar to that of responses to GABA. This was true for both mono- and biphasic responses to the agonists.

Isoguvacine is another GABA analogue with a weak affinity for the GABA carrier (Lodge *et al.* 1978). When administered on seven cells by drop, isoguvacine was 0·5–100 times (mean 40 times) as potent as GABA but the time course of equiamplitude responses was indistinguishable from those to GABA and muscimol.

Is GABA uptake related to desensitization? In the central nervous system, inhibition of GABA uptake increases not only the amplitude but also the time course of responses to exogenous GABA (Curtis *et al.* 1976). In the DRG the effects of uptake inhibition

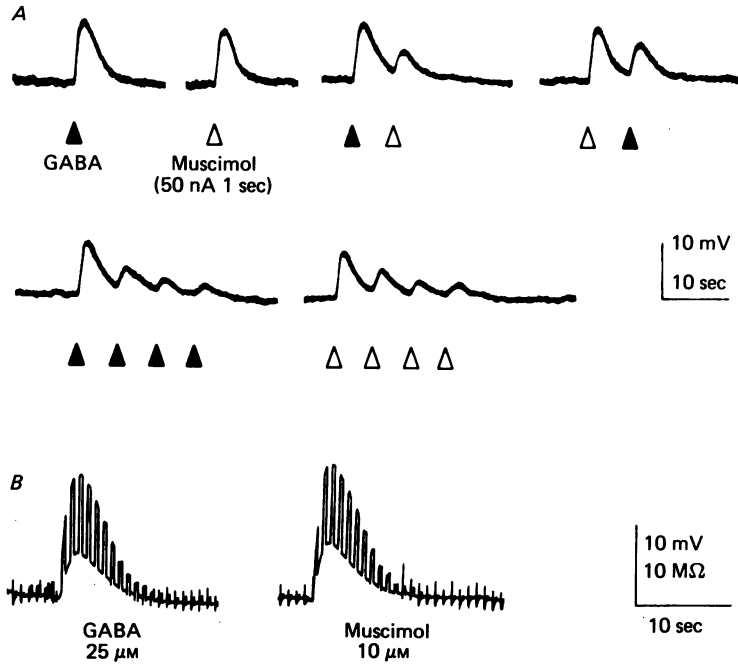

Fig. 5. Comparison of the effects of GABA and muscimol on DRG neurones. *A*, responses to the two agonists were of a similar time course and displayed full cross-desensitization, as shown in the upper row of traces. Thus muscimol, administered just after the end of the response to GABA, evoked a smaller response than when ejected on its own; similarly GABA after muscimol evoked a reduced response. Repeated ejections of the two agonists (middle traces) show that muscimol is as prone as is GABA to desensitization. The gaps in the record are each of 1 min. Resting potential −64 mV; input resistance 5 MΩ. *B*, records from another DRG neurone illustrating responses elicited by a drop of each agonist placed into the superfusion stream. Note the similarity of the response characteristics but the greater potency of muscimol. Hyperpolarizing current pulses of 1 nA, 300 msec. Cell resting potential −66 mV; input resistance 17 MΩ.

cannot be examined in the same way on the duration of responses to GABA because of the rapid desensitization of the responses. On the other hand the relation of uptake to this desensitization process can be studied: if the onset of 'desensitization' really is related to changing extracellular GABA levels caused by a delayed activation of uptake processes (Krnjević *et al.* 1977) then inhibition of uptake should alter both the amplitude of the second phase of biphasic responses to GABA and the rate of onset of the 'desensitization' process. We have examined this possibility by studying the size of the second phase of responses to GABA during uptake inhibition with

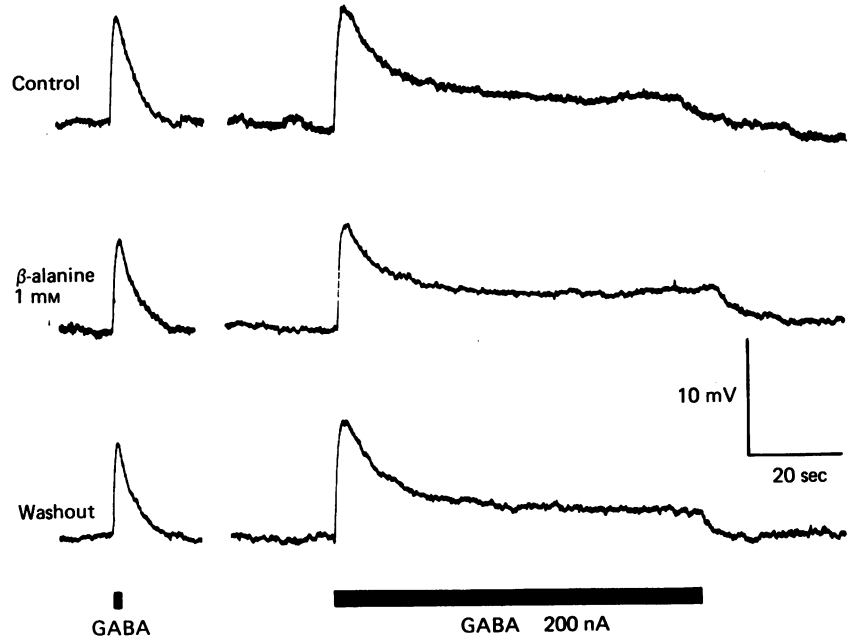

Fig. 6. Lack of effect of superfusing 1 mM-β-alanine on mono- and biphasic GABA-mediated depolarizations of a DRG neurone. After 35 min of β-alanine there was no clear effect on the amplitude or on the time course of either phase of the response. Resting potential −58 mV.

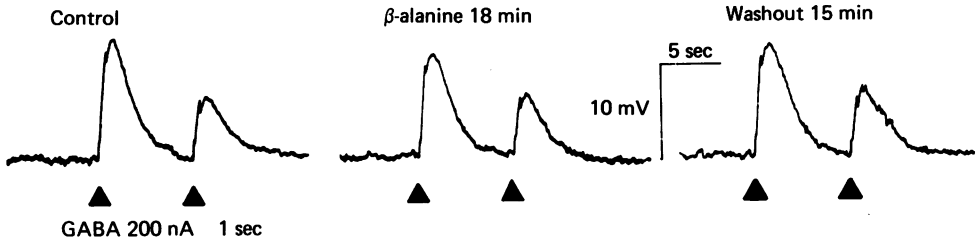

Fig. 7. Lack of effect of an 18 min superfusion of 1 mM-β-alanine on the time course of desensitization to ionophoretic GABA. In each of the three traces an initial control response is followed (interval 12 sec) by a test response which, because of the desensitization, is only about half the size of the control response. Superfusion of β-alanine did not affect the proportionate reduction of the test response, indicating that the time course of desensitization was not affected. Resting potential −73 mV; input resistance 8 MΩ.

β-alanine (eleven cells), chlorpromazine (one cell) and zero sodium (eight cells). In no case was there any marked change in the second phase. In the example of Fig. 6, superfusion of β-alanine for 35 min had no clear effect on either mono- or biphasic responses to GABA.

One method of studying the time course of desensitization is to evoke two short GABA responses about 10 sec apart and to express the amplitude of the second response as a proportion of the first: any change in the time course of desensitization should be reflected in a change of this proportion. Ten cells were studied in this way

with β-alanine (duration of superfusions 7–19, mean 13 min). In no case was a clear change in the relation of first and second responses seen during uptake inhibition (Fig. 7).

GABA causes desensitization at micromolar concentrations. The experiments described above indicate that the desensitization of GABA receptors cannot be explained in terms of an activation of GABA uptake, and must therefore be a true receptor phenomenon. It is of relevance, in determining the possible physiological significance of such desensitization, to know what are the lower limits of GABA concentrations which can cause it.

GABA was therefore added to the superfusion medium at 1–5 μM whilst ionophoretic responses to GABA were elicited regularly. GABA 1 μM (duration 10–30, mean 19 min) slightly reduced the amplitude of ionophoretic responses of one of six cells tested; 2 μM (6, 18 min) reduced responses of one of two neurones and 5 μM caused a 10–40 % reduction on seven of eight cells (eight to twelve, mean 10 min superfusions).

DISCUSSION

The principal conclusions to be drawn from our experimental results are firstly that glial uptake of GABA affects neither the amplitude nor the time course of the responses of larger-diameter DRG neurones to GABA, and secondly that desensitization of mammalian GABA receptors can occur independently of GABA uptake.

The *in vitro* preparation employed necessarily precluded any identification of cells on a physiological basis. As a result our sample of neurones no doubt included various functional cell types amongst the population of larger DRG neurones which were selected for study. This heterogeneity may explain some of the variation in cell properties, such as the presence and form of action potentials (see also Yoshida & Matsuda, 1979). It could also explain the variation of input resistance, but the fact remains that this large variation is in marked contrast to the very small variability, about a mean of 5 MΩ, seen in a similar study of cat DRG *in vitro* (Gallagher *et al.* 1978). In both studies single barrel micropipettes were used so that care must be taken in the interpretation of resistance measurements; nonetheless it seems improbable that the disparity between the two studies is solely artifactual. Because in our study the cells were impaled under direct vision, we can say that the variation was not due only to difference in cell size, although the smallest cells impaled did have above-average input resistances.

It is evident from our results that, as expected, the resting chloride conductance was low. More surprisingly our experiments suggested that appreciable chloride movement could occur when GABA was administered frequently, even when applications were for only 1 sec every 45–90 sec. Whilst direct measurements of the GABA reversal potential would be needed to prove this point, the important finding in our experiments was that the reduction of responses to GABA during low chloride could be followed by a transient inversion of responses to GABA (i.e. from depolarizing to hyperpolarizing) after replacement of normal chloride. This indicated that there was a marked alteration of intracellular chloride levels (as also seen by Adams & Brown, 1975); the results cannot be explained by the possibility that 'foreign anions may have a direct pharmacological action when substituted for chloride in the

extracellular solution' (Gallagher *et al.* 1978), since in the latter case an inversion of the responses to GABA could not occur. Nor does it seem likely that the temperature used in our experiments (30–35 °C) was too low to maintain normal chloride pumping since Adams & Brown (1975) recorded reproduceable responses from sympathetic ganglion cells kept at 16–24 °C, although it may be relevant that in this latter study the estimate of E_{GABA} was a more negative value than other estimates performed at 37 °C. (it should be pointed out that experiments with inhibitors of the chloride pump appear not to be practical on DRG neurones, for Nicoll (1978) has found that in the frog the available inhibitors interact with chloride channels and hence have direct effects on responses to GABA).

Glial uptake inhibition and response amplitude. β-alanine inhibits glial uptake of GABA competitively by riding the same carrier; it also hetero-exchanges with GABA (see Iversen & Kelly, 1975). That is to say β-alanine will, as well as inhibiting uptake, also induce a release of GABA from the satellite glial cells. The normal GABA content of DRG is low (in comparison with tissues in the central nervous system; see review by Curtis, 1978) but after a few administrations of GABA the glia around the neurone under study may accumulate sufficient GABA for hetero-exchange to become appreciable. Indeed biochemical experiments have shown that both β-alanine and sodium-free solutions do release preloaded GABA from DRG (Minchin & Iversen, 1974; Minchin, 1975). Chlorpromazine has not been tested for effects on GABA efflux from DRG, but by inference from results obtained with rat cortical tissue (Olsen, Ticku, van Ness & Greenlee, 1978) it probably decreases, rather than enhances, the release of GABA.

It also seems that these uptake-inhibiting procedures affected DRG neurones directly, in spite of the fact that resting membrane potential and conductance were not affected to any great extent. For example, β-alanine (1 mM) reduced responses to GABA on some occasions and this concentration therefore appears to be threshold for affecting the GABA receptors of these neurones. This suggestion is supported by the finding that a tenfold higher concentration of β-alanine rapidly reduced responses to GABA, and is consistent with the results of binding studies on brain tissue (e.g. Hitzemann & Loh, 1978).

Although responses to GABA are mediated principally if not exclusively by enhancing chloride conductance, there are reports that reduced extracellular sodium levels have complex effects on such responses, at least those of frog primary afferent neurones and terminals (Barker & Nicoll, 1973; Nishi *et al.* 1974). We too have occasionally seen complex effects of sodium-free solutions on responses to GABA. Whilst we have not investigated these effects further, it is our impression that they were always associated with some degree of depolarization and a change in membrane conductance. The possibility exists that these effects were at least partly due to the Tris in the superfusate.

It was because of these various complications that we felt it to be important to test for effects of glial uptake inhibition by the three different means. The consistent lack of effect of glial uptake inhibition on responses to GABA provides compelling evidence that under our experimental conditions GABA uptake cannot determine response amplitude or duration. One particular problem in drawing this conclusion was the following possibility: that response potentiation by uptake inhibition could

be balanced out by a mild desensitization resulting from increased GABA efflux during uptake inhibition by β-alanine or zero sodium. It seems inconceivable, however, that these two opposing forces should so frequently just balance each other. Moreover, chlorpromazine probably does not cause a release of GABA, and yet this inhibitor did not potentiate responses either, with the exception of a small effect on two cells, a result scarcely consonant with a significant effect by glial uptake on response amplitude.

The question of the concentration of GABA attained in our experiments is important for three principal reasons. First, the autoradiographic localization of radio-labelled GABA in satellite glia was established at a concentration of 1–5 μM-GABA (Schon & Kelly, 1974*a*). Secondly, the properties of the uptake inhibitors were determined by Schon & Kelly (1974*b*) at a concentration of 0·1 μM-GABA. Thirdly, Brown & Galvan (1977) found that uptake inhibition potentiated the responses recorded from the surface of superior cervical ganglia only when the concentration of GABA did not exceed 10 μM, i.e. about 50 % more than the K_m of the high affinity uptake of GABA by glial cells (K_m 7 μM). In dorsal root ganglia Schon & Kelly (1974*b*) found an apparent K_m of 10 μM.

It therefore seems that the concentrations of GABA (3–20 μM) eliciting threshold responses recorded intracellularly in our system were, at least on some cells, sufficiently low not to saturate the high affinity uptake system. These values of concentrations were, however, estimated indirectly and in addition may not be entirely comparable with the concentrations attained with micro-electrophoretic administrations. In this regard it is pertinent to point out again that the ionophoretic pipettes were upstream from, and not in contact with, the cell under study, so that very localized, very high concentrations of GABA should have been avoided.

An explanation is thus required for the apparent inconsistency between our negative results and the positive effects of uptake inhibition seen on sympathetic ganglia by Brown & Galvan (1977). The considerable methodological differences between these studies offer at once difficulties of comparison and a possible explanation of the discrepancy. First in the study on sympathetic ganglia the superfusions of GABA were long (4 min) relative to the onset of desensitization (⩽ 30 sec; Adams & Brown, 1975). Secondly, Brown & Galvan (1977) saw the greatest enhancement with a concentration of only 1 μM-GABA, which was, as discussed above, below the lower limit of GABA concentrations eliciting responses readily detectable by intracellular recording. Thirdly, it is probable that inhibition of glial uptake of GABA could enhance surface potentials by increasing the diffusion of GABA into the ganglion, so that a greater proportion of neurones in the ganglion will contribute to this depolarization. In other words uptake inhibition could enhance surface potentials without necessarily affecting the responses of neurones close to the source of GABA. Indeed we have evidence to support this suggestion, for although we saw no potentiation of responses of surface cells recorded intracellularly, Brown & Desarmenien, in preliminary experiments, did see an enhancement of surface potentials recorded from DRG, although this potentiation was less marked than with sympathetic ganglia.

This finding suggests that the structural differences between superior cervical and dorsal root ganglia are not the main reason for the apparent discrepancy. The presence of presynaptic fibres and terminals in sympathetic ganglia may nonetheless

be significant, particularly since Brown & Marsh (1978) have recently shown that many unmyelinated fibres possess receptors for GABA.

The lack of effect of uptake inhibition on the amplitude of the second phase of response to GABA indicated that this plateau is not an indirect effect of uptake, a possibility since the depolarization could be caused by the increased extracellular potassium which occurs simultaneously (Deschenes & Feltz, 1976) and which could result from a sodium-coupled glial transport of GABA (see also Hösli Andrès & Hösli, 1978).

Responses to muscimol and isoguvacine. The similarity of the responses to GABA, muscimol and isoguvacine supports the idea that uptake does not affect GABA actions under our conditions, for muscimol and isoguvacine are probably not transported by the glial GABA carrier (Johnston *et al.* 1978; Schousboe, Krogsgaard-Larsen, Svenneby & Hertz, 1978). It should be borne in mind, however, that any tendency for muscimol and isoguvacine to have a prolonged action on DRG neurones may be negated by the onset of desensitization; the action of muscimol is indeed very long on cat primary afferent terminals (Curtis & Lodge, 1978) where desensitization is less evident.

GABA uptake and desensitization. Although there is clear evidence from non-mammalian preparations for a true desensitization of GABA receptors, Krnjević *et al.* (1977) have suggested that the apparent desensitization seen in their *in vivo* records of mammalian central neurones is the result of a GABA-induced activation of GABA uptake: this was postulated to result in a reduction of extracellular GABA concentration and hence a diminution of the GABA-induced conductance change; electrogenic uptake into the neurones themselves would complicate the final potential changes.

If this were also the case in the DRG then inhibition of uptake should have prevented the apparent desensitization. On the contrary, however, uptake inhibition had no effect on the amplitude of the second phase of biphasic responses, nor on the reduction of the GABA-induced conductance increase, nor on the proportionate reduction of the second of two adjacent short responses. In addition muscimol and isoguvacine display desensitization just as does GABA. These findings indicate clearly that the GABA desensitization seen in DRG is a receptor phenomenon rather than being an effect secondary to uptake activation.

Possible implications of desensitization. The findings that receptors for GABA can be partially desensitized by concentrations of GABA as low as 1 μM has possible physiological implications, particularly if such concentrations also affect GABA receptors in the central nervous system. The levels of GABA in cat plasma have been estimated to be 2–4 μM (Crowshaw, Jessup & Ramwell, 1974) although, as Brown & Galvan (1977) have pointed out, the interstitial concentration of GABA may be considerably lower than this, due to uptake. Nonetheless it is quite possible that micromolar concentrations could result from release of GABA induced by potassium concentrations reached under *in vivo* conditions of high neuronal activity. Any resultant desensitization would result in a positive-feedback situation which could, for example, contribute to the spread of epileptiform activity. Such a possibility clearly needs further investigation.

Conclusion. The present findings indicate clearly that in dorsal root ganglia, glial

uptake of GABA is not related to the onset of desensitization, nor does it affect the time course or the amplitude of responses of neurones adjacent to the source of GABA. The apparent discrepancy between this negative finding and the enhancement, during uptake inhibition, of surface potentials recorded from sympathetic ganglia may be explicable in terms of diffusion of GABA through the ganglia, as discussed above. The same reasoning cannot, however, explain the potentiation seen in the central nervous system (Curtis *et al.* 1976; Lodge *et al.* 1977). An alternative explanation may lie in the durations of the responses examined: thus ionophoretic administrations of GABA over several seconds (Curtis *et al.* 1976; Lodge *et al.* 1977) may affect a sufficient volume of tissue for uptake, as well as simple diffusion, to affect the distribution of GABA; whereas GABA released either synaptically or by *short* ionophoretic pulses may decline in concentration too rapidly by simple diffusion for uptake to have any effect (see also Curtis *et al.* 1976). The lack of effect of uptake on prolonged responses to GABA in DRG is not inconsistent with such a hypothesis simply because the rapid desensitization of GABA receptors precludes observing any more slowly occuring phenomena. From these points of view, our experimental conditions may, paradoxically, mimic synaptic release more closely than other studies have done, in that the GABA administrations were relatively short ($\leqslant 250$ msec), were usually from a point source, and were close to the neurone under study. Indeed, uptake inhibition failed to affect not only responses to the fairly short GABA administrations in the present study, but also the even shorter synaptic inhibitions tested in the central nervous system (Curtis *et al.* 1976; Lodge *et al.* 1977). Other aspects must however be considered, in particular the fact that in the central nervous system most GABA uptake is probably by neuronal elements (see Iversen & Kelly, 1975); there also remains the possibility that the negative result obtained with tests on central inhibitions is due only to failure of the uptake inhibitors to reach synaptic regions (Curtis *et al.* 1976). Nonetheless the available evidence is consistent with the notion (see also Brown & Galvan, 1977; Lodge *et al.* 1978) that diffusion of GABA from receptors is sufficiently rapid to account for the form of short-lasting responses to GABA and that uptake only affects response characteristics when the continued administration of GABA affects the local diffusion gradients.

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