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γ-AMINOBUTYRIC ACID EFFLUX FROM SYMPATHETIC GLIAL CELLS: EFFECT OF 'DEPOLARIZING' AGENTS

BY N. G. BOWERY*, D. A. BROWN AND S. MARSH

From the Department of Pharmacology, the School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX and the Department of Pharmacology, St Thomas' Hospital Medical School, London SE1*

WITH AN APPENDIX BY P. R. ADAMS† AND D. A. BROWN

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SUMMARY

1. Isolated desheathed rat superior cervical ganglia were incubated in [^{3}H]2,3-γ-aminobutyric acid ([^{3}H]GABA) solution (1–10 μM for 2–3 hr) in the presence of 10 μM-amino-oxyacetic acid (AOAA). The subsequent efflux of tritium into a stream of superfused non-radioactive GABA-free Krebs solution at 25 °C was measured.

2. In the presence of 10 μm-AOAA the mean basal efflux rate coefficient (k_0) for exit of tritium into the superfusion fluid was $0{\cdot}7 \times 10^{-3}$ min^{-1}. More than 98% of effluent tritium comprised unchanged [^{3}H]GABA. The rate coefficient showed no correlation with the amount of [^{3}H]GABA previously accumulated by the ganglion.

3. Elevation of $[K^+]_0$ to > 50 mM increased the rate coefficient for [^{3}H]GABA release by up to four times. Changes in efflux rate were not correlated with osmotic changes, and persisted after re-accumulation of effluent [^{3}H]GABA by the inward carrier was inhibited. The effect of alkali metal cations diminished in the order $Rb^+ > K^+ > Cs^+Li^+$. Effects of K^+ solutions were not reduced by omitting Ca^{2+} ions, with or without the addition of Mg^{2+}.

4. Application of electrical pulses (0·1–1 msec duration, 1–10 Hz, 4 min trains) to the ganglion soma or to the preganglionic nerve trunk also raised k_0. This effect declined with repeated stimulus trains, without an accompanying diminution in the response to K^+. Responses to electrical stimulation were not reduced by amethocaine (300 μM), tetrodotoxin (3 μM) or raised $[Mg^{2+}]$ (0 mM-$[Ca^{2+}]$/30 mM-$[Mg^{3+}]$). Separate local superfusion of the pre- and post-ganglionic nerve trunks and of the ganglion soma showed that the response to electrical stimulation was localized to the vicinity of the stimulus and was not propagated along the nerve trunks or across the synapses.

5. Electrical recording from impaled 'inexcitable' cells (presumed to be neuroglial cells (Appendix)) indicated that the quantities of K^+ ion accumulating during repetitive nerve stimulation are insufficient to stimulate the release of GABA from the glial cells. No physiological role for the release process in modulating neuronal excitability could be adduced.

† Present address: Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.

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INTRODUCTION

Neuroglial cells in mammalian sympathetic ganglia take up radioactively labelled γ-aminobutyric acid (GABA) by a carrier-mediated transport system (Bowery & Brown, 1972; Young, Brown, Kelly & Schon, 1973). The characteristics of the influx process have been described in some detail in the preceding paper (Bowery, Brown, White & Yamini, 1979).

The present paper concerns the efflux of [^{3}H]GABA from preloaded rat superior cervical ganglia. We have previously reported (Bowery & Brown, 1972) that this efflux is slow, with a rate coefficient around 10^{-3} min^{-1}, but can be accelerated by raising the K^+ concentration of the external medium, or by passing an electric current across the tissue. We have undertaken a more detailed study of the effect of these 'depolarizing' stimuli, primarily with the aim of finding out whether it reflects some property of the efflux process which could be of physiological significance. A brief account of some of these experiments has been given (Bowery & Brown, 1974*b*). Some relevant information concerning the electrical responses of presumed neuroglial cells to K^+ ions and to orthodromic stimulation is presented in the Appendix.

METHODS

Desheathed superior cervical ganglia were dissected and maintained in Krebs solution as described in the preceding paper (Bowery *et al.* 1979). Unless otherwise stated, experiments were performed at 25 °C in the presence of 10 μM-amino-oxyacetic acid (AOAA). Ganglia were 'loaded' with radioactively labelled GABA by incubating them in 1–10 μM [^{3}H] GABA for 2–3 hr, and the subsequent efflux of radioactivity into non-radioactive Krebs solution superfused over the ganglion measured at 2 min intervals in the manner described in a previous publication (Bowery, Brown, Collins, Galvan, Marsh & Yamini, 1976). Two different types of superfusion system were used. (*a*) In the majority of experiments, the preparation was mounted vertically so that the ganglion and its attached pre- and post-ganglionic nerve trunks were simultaneously superfused with the same solution, at a constant rate of 0·5 ml. min^{-1}. The superfusion chamber (see inset to Fig. 11) contained three pairs of indwelling bipolar platinum electrodes, such that the ganglion soma itself or either nerve trunk could be stimulated separately. The post-ganglionic response to pre-ganglionic stimulation was recorded on an oscilloscope. (*b*) To determine from which segment of the preparation the effluent radioactivity stemmed, an alternative arrangement was used in some experiments whereby the preparation was mounted *horizontally* in a triple-chambered bath (Bowery & Tulett, 1975), likewise containing three arrays of electrodes for stimulating the pre- or post-ganglionic trunks or the ganglion soma. Each chamber was then perfused individually, so that the efflux of radioactivity from each segment of the preparation could be measured separately. Total effluent radioactivity was measured at 2 min intervals and the efflux expressed as the rate coefficient (Bowery *et al.* 1976) after measuring the amounts of radioactivity left in the tissue at the end of the experiment. Experiments were undertaken on more than 100 preparations; unless otherwise stated, observations described in the results were confirmed in three or more preparations.

Identification of effluent radioactivity. Radioactivity collected in the superfusion fluid was normally too low to permit 'on-line' identification and separation of labelled GABA. Instead, checks were made in separate experiments in which the effluent radioactivity from several ganglia pre-incubated in [^{14}C]GABA was collected over several hours, concentrated by evaporation to dryness, redissolved in a small volume of deionized water and subjected to paper electrophoresis under a gradient of 20 V/cm. ^{14}C-distribution was measured using a radiochromatogram scanner and GABA located with ninhydrin. Table 1 shows the results of these experiments. The ganglia were loaded in the presence of AOAA and efflux was measured in the absence or presence of AOAA, under two experimental conditions – in normal Krebs solution (5·9 mM-[K^+]) and in

a solution containing 152 mM-[K^+], added in the form of K_2SO_4. Effluent radioactivity measured in the presence of AOAA was unchanged [^{14}C]GABA in both high and low [K^+] solutions. In the absence of AOAA nearly all of the radioactivity released into 5·9 mM-[K^+] solution consisted of metabolites, whereas radioactivity released into 152 mM-[K^+] was unchanged [^{14}C]GABA. In all cases residual radioactivity in the ganglion was unchanged [^{14}C]GABA or substantially so.

TABLE 1. Paper electrophoretic analysis of released [^{14}C]GABA measured in the presence (+) and absence (−) of amino-oxyacetic acid (AOAA, 10 μM), (*a*) in normal Krebs solution containing 5·9 mM-[K^+] and (*b*) in Krebs solution containing 72 mM-K_2SO_4 substituted for NaCl (152 mM-[K^+]). In each experiment four ganglia were incubated for 4 hr in 5 μM-[^{14}C]GABA in the presence of 10 μM-AOAA. They were then transferred to 4 ml. of the appropriate solution bubbled continuously with 95% O_2/5% CO_2 mixture for 17 hr in (*a*) and 3·3 hr in (*b*). After incubation ^3H was extracted from the ganglia by homogenizing them in 200 μl. ice-cold deionized water, and from the incubation medium by evaporating to dryness and redissolution in 200 μl. deionized water. Solutions were subjected to paper electrophoresis and ^{14}C distribution measured with a radiochromatograph scanner

	Effluent medium			
	(*a*) 5·9 mM-[K^+]		(*b*) 152 mM-[K^+]	
Radioactivity	+AOAA	−AOAA	+AOAA	−AOAA
(1) Total left in ganglion (nc)	22·5 (85%)	10·5 (47%)	50 (25%)	36 (25%)
(2) Total in effluent medium (nc)	4·1 (15%)	11·9 (53%)	152 (75%)	107 (75%)
(3) (1)+(2) (nc)	26·6	22·4	202	143
(4) % [^{14}C]GABA in ganglion	98%	98%	98%	80%
(5) % [^{14}C]GABA in medium	98%	5%	98%	98%

Osmotic effects of K^+ solutions. Changes in tissue water content, and in intracellular and extracellular fluid volumes, produced by K^+ solutions, were measured in the manner described by Brown, Halliwell & Scholfield (1971). Paired (left and right) ganglia were pre-equilibrated for 60 min in Krebs solution at 25 °C bubbled with 95% O_2/5% CO_2 containing 10 μc.ml.$^{-1}$ [^3H]inulin. One ganglion was then transferred to the appropriate K^+ solution for testing (also containing [^3H]inulin), the other to a matching solution of normal Krebs, for a further 15 min. The ganglia were then withdrawn, rinsed briefly, weighed, dried for 1 hr at 85 °C, reweighed and assayed for tritium (see Brown *et al.* 1971, for details). Total water content (W_T) was calculated as fresh weight less dry weight, and converted to l. volume assuming unit density. 'Extracellular' water (W_E) was expressed as the volume of distribution of [^3H]inulin (l.) and 'intracellular' water (W_I) calculated as $W_T - W_E$. Water spaces were expressed as multiples of dry weight, and effects of the K^+ solution calculated between paired ganglia as (test minus control)/(test), expressed as percentage change. In some experiments 1 μc.ml.$^{-1}$ [^{14}C]urea was added to all solutions and W_T measured as the urea space. This is equal to wet weight less dry weight, corrected to volume at unit density (Garthwaite, 1977), and the $^3H/^{14}C$ ratio provided an additional check for changes in fluid distribution within the tissue. Water spaces were then expressed as multiples of the non-urea space (= fresh weight (volume) less urea space).

Sources of materials. [^3H]GABA [2,3 ^3H]4-amino-*n*-butyric acid (10 c.m-mole^{-1}), [4,5 ^3H]L-leucine (42·7 c.m-mole^{-1}) and [methyl ^3H]choline (2 c.m-mole^{-1}) were obtained from N.E.N. GmbH. [^3H]inulin (1·09 c.m-mole^{-1}), [^{14}C]urea (59 mc.m-mole^{-1}) and a second batch of [4,5 ^3H]L-leucine (60 c.m-mole^{-1}) were obtained from the Radiochemical Centre. The ionophores

X537-A and A23187 were supplied by Hoffmann-La Roche and Lilly Research Laboratories (Eli Lilly & Co.) respectively.

RESULTS

The rate coefficient for [^{3}H]GABA efflux (measured in the presence of AOAA) assumed a fairly constant level in each experiment after 30–60 min washing, varying between experiments over the range $0{\cdot}2–3 \times 10^{-3}$ min^{-1}. There was no correlation between the absolute rate coefficient and the concentration of [^{3}H]GABA initially

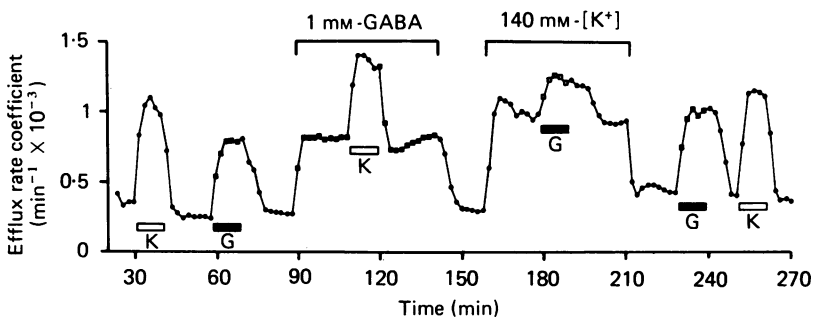

Fig. 1. Effect of raising external [K$^+$] from 6 to 140 mM (by adding 134 mM-KCl: at K) and of adding 1 mM unlabelled GABA (at G) on the efflux of [^{3}H]GABA from a superfused rat superior cervical ganglion. The ganglion had been previously loaded with [^{3}H]GABA by incubation for 3 hr in 1 μM-[^{3}H]GABA. It was then washed for 60 min in non-radioactive Krebs solution before beginning the superfusion. Both incubation and superfusing fluids contained 10 μM-amino-oxyacetic acid. The time scale (abscissae) gives the time after starting the superfusion. The efflux rate (ordinates) is expressed as the rate coefficient (min$^{-1} \times 10^{-3}$), measured at 2 min intervals. The concentration of unlabelled GABA used saturates the inward carrier and hence eliminates re-uptake (see Bowery *et al.* 1976); notwithstanding, the accelerative effect of K$^+$ ions is unchanged.

present in the tissue (which ranged from 0·03 to 0·36 mM). This accords with the results of previously described experiments (Bowery *et al.* 1976) in which the levels of endogenous GABA in the tissue were deliberately elevated (from 0·21 to 0·73 mM) without a corresponding change in the rate coefficient for efflux of [^{3}H]GABA. In the absence of AOAA the efflux rate of labelled material (principally metabolites, see Table 1), was 10–20 times faster. All experiments described below were performed in the presence of AOAA, so that measured radioactivity refers to unchanged [^{3}H]GABA.

The release of [^{3}H]GABA by K$^+$ ions

Addition of K$^+$ ions (as KCl or K_2SO_4) increased the rate of [^{3}H]GABA efflux (Fig. 1). Efflux was maximally accelerated within 4 min of adding the K$^+$ solution and was sustained for periods of at least 60 min. The peak efflux rate coefficient was linearly dependent on $[K^+]_o$ within the range 70–160 mM, attaining a value about 4 times the resting value at the highest concentration used (Fig. 2*A*); there was little change in efflux rate below 50 mM-[K$^+$].

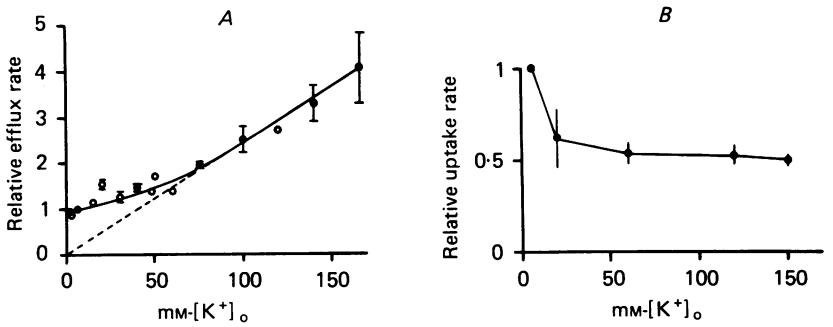

Fig. 2. [^{3}H]GABA efflux rate (*A*) and uptake rate (*B*) measured in solutions containing different concentrations of K^+ (as added KCl), and expressed as a fraction of that in normal Krebs solution ($[K^+]_o$ = 6 mM). Filled circles represent the means of two (*B*) or three or more (*A*) measurements: bars represent standard errors (*A*) or range (*B*); open circles refer to single measurements. Efflux rates refer to the peak rate coefficient attained after 4–10 min; uptake rates refer to amounts of [^{3}H]GABA accumulated after 10 min incubation in 0·5 μM-[^{3}H]GABA, corrected for extracellular infiltration (see Bowery *et al.* 1979).

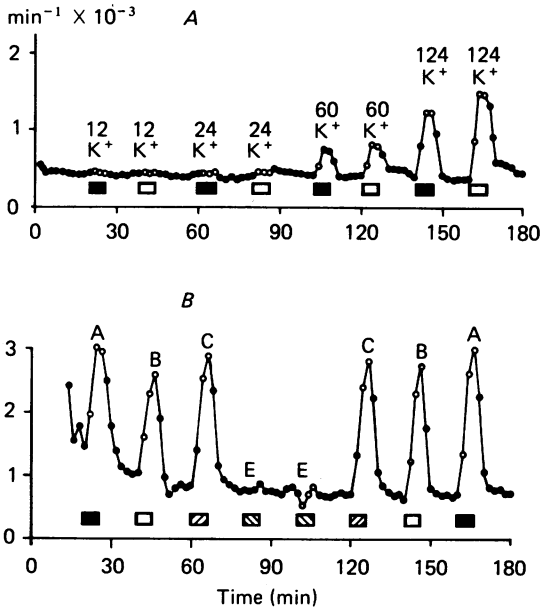

Fig. 3. Effects of K^+ solutions of different osmolarity on the rate coefficient for [^{3}H]GABA efflux. In *A* $[K^+]_o$ was raised to 12, 24, 60 and 124 mM by *either* addition of KCl to normal Krebs solution (= 6 mM-$[K^+]_o$) (■) *or* substitution of KCl for NaCl (□). In (*B*), $[K^+]_o$ was raised to 124 mM in A, B and C by addition of KCl to normal Krebs solution (A, ■), or substitution of 118 mM-KCl (B, □) or K isethionate (C, ▨) for NaCl. Solution E (▧) contained 118 mM-Tris HCl substituted for NaCl. The detailed composition of solutions A, B, C and E are given in Table 2; osmotic effects of A, B and C are shown in Fig. 4. (The pH of the Tris-HCl solution was adjusted to 7·3 by adding 15 mM-Tris-base.)

Osmotic effects of K^+ salts

The acceleration of GABA efflux shown in Figs. 1 and 2 could not be attributed to the osmotic effects of adding the K^+ salts to the Krebs solution since they were replicated (up to 124 mM-[K^+]) by *substituting* KCl or K isethionate for NaCl (Fig. 3). Substitution of Tris-Cl for NaCl, to produce an equal reduction in [Na^+] from 143 to 25 mM), had no clear effect on [^{3}H]GABA efflux (solution E in Fig. 3*B*).

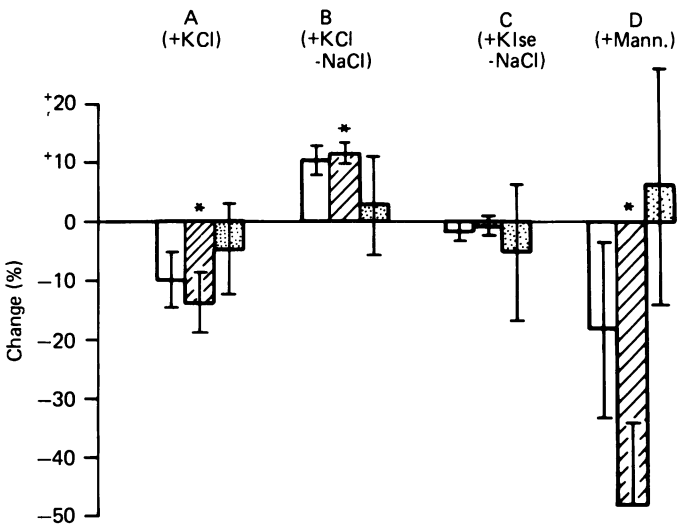

Fig. 4. Effects of 15 min incubation in four different solutions on the apparent water-spaces of isolated ganglia, measured as described in Methods. Clear columns, total water; striped columns, intracellular water; stippled columns, extracellular water. Ordinates show mean % changes over paired controls in normal Krebs solution; bars show standard errors of means (n = 4–7); * significant change from paired controls ($P < 0.05$; two-tailed t test). The composition of solutions A (added KCl), B (KCl substituted for NaCl), C (K isethionate substituted for NaCl) and D (added D-mannitol) are given in Table 2.

Addition or substitution of KCl produced *opposite* effects on the total intracellular fluid spaces of the ganglion, as shown in columns A and B respectively of Fig. 4. Substitution of K isethionate for NaCl appeared to preserve the fluid volumes quite well (column C). Addition of 216 mM-D-mannitol (the approximate osmotic equivalent of adding 118 mM-KCl: Robinson & Stokes, 1955) produced very considerable cell shrinkage (column D of Fig. 4), and hence could not be used as an osmotic 'control' for KCl.

The osmotic effects shown in Fig. 4 agree qualitatively with those anticipated if the cell membranes in the ganglion were permeant to K^+ and Cl^- ions, but not to Na^+, isethionate or mannitol. Quantitatively, the cell shrinkage in mannitol solution is not very different from that expected from eqn. (13a) of Boyle & Conway (1941) (see Table 2), but the apparent swelling in KCl-substituted solution is a lot less than expected. In part this may reflect incomplete equilibration of K^+, Cl^- and water across the cell membranes after a relatively brief incubation period, and much larger increases in apparent intracellular volume have been previously observed in this tissue after more prolonged (2–3 hr) immersion in solutions in which NaCl was substituted

by KCl (+130 %, Brown & Halliwell, 1972 and unpublished measurements; +40 %, Garthwaite, 1977). Also, it is unlikely that the permeability of the ganglionic *neurones* to Cl^- ions is as high as their permeability to K^+ (see Adams & Brown, 1975). The relative Cl^- and K^+ permeabilities of ganglionic glial cells are unknown, though P_{Cl} appears to be much less than P_K in other glial cells (Kuffler, Nicholls & Orkand, 1966; Dennis & Gerschenfeld, 1969; Ransom & Goldring, 1973*a*). The essential point is that, irrespective of the relative permeabilities to Cl^- and K^+, the volume changes produced by addition or substitution of KCl should be, and were, very different, whereas their effects on [³H]GABA efflux were similar.

TABLE 2. Composition of solutions (mM) used to stimulate [³H]GABA efflux in Fig. 3*B*. (Osmotic effects are shown in Fig. 4)

	Solution					
	Normal (NK)	A	B	C	D	E
NaCl	118	118	0	0	118	0
Tris (Cl)	0	0	0	0	0	118
$NaHCO_3$	25	25	25	25	25	25
KCl	4·7	123	123	4·7	4·7	4·7
K isethionate	0	0	0	118	0	0
KH_2PO_4	1·2	1·2	1·2	1·2	1·2	1·2
$CaCl_2$	2·5	2·5	2·5	0·6	2·5	2·5
Ca acetate	0	0	0	1·3	0	0
$MgSO_4$	1·2	1·2	1·2	1·2	1·2	1·2
D-mannitol	0	0	0	0	216	0
$[Na^+]_o$	143	143	25	25	143	25
$[K^+]_o$	6	124	124	124	6	6
$[Cl^-]_o$	128	246	128	6	128	128
$[K^+]_o \times [Cl^-]_o$	753	30,504	15,872	753	753	753
*C_{osm}	330	549	328	330	549	345§
† V	1·0	0·98	2·37	1·0	0·59	0·95§
‡ v	(1·0)	0·86	1·11	0·99	0·52	

* Total osmotic solute concentration (m-osmole.$l.^{-1}$).

† Relative intracellular volume assuming K^+ and Cl^- permeability, calculated from eqn. (13*a*) of Boyle & Conway (1941) expressed in the form

$$V = \frac{V_x}{V_{NK}} = \frac{C_{osm(x)}}{C_{osm(NK)}} \cdot \left[\frac{C^2_{osm(NK)} - 4[K^+_{NK}]_o\,[Cl^-_{NK}]_o}{C^2_{osm(NK)} - 4\,[K^+_X]_o\,[Cl^-_X]_o}\right],$$

where the subscripts X and NK refer to normal Krebs and test solution X respectively.

‡ v = observed relative intracellular fluid volume (from data in Fig. 4).

§ 15 mM-Tris-base added to pH 7·3.

[¹⁴C]urea efflux. In agreement with the above conclusion, addition of 134 mM-KCl (to give 140 mM-$[K^+]_o$: three experiments, five test applications) did not consistently alter the rate of [¹⁴C]urea efflux from preloaded ganglia. Addition of 68 mM-K_2SO_4 (one experiment) increased the rate coefficient for urea efflux by about 40 %, much less than that for [³H]GABA efflux. (The rate coefficient for urea efflux fell to a minimum value of 0·05–0·1 min^{-1} during continuous superfusion, i.e. about 100 times greater than that of GABA: this renders urea of somewhat limited use as a control for GABA fluxes.)

Effect of K^+ salts on re-uptake

Efflux of [^{3}H]GABA from this tissue appears to be subject to a continuous process of re-uptake from the restricted interstitial spaces, so that the *observed* efflux of [^{3}H]GABA into GABA-free solution represents only a minor fraction (about 20–30 %) of the true transmembrane flux (Bowery *et al.* 1976). Since elevation of $[K^+]_o$ reduces the uptake rate (Bowery *et al.* 1979), inhibition of re-uptake might therefore contribute to the observed increase in measured efflux rate. This effect appears to be of minor importance to the action of K^+ ions for the following reasons.

(*a*) There are substantial differences between the effects of different K^+ concentrations on influx and efflux (compare *A* and *B* in Fig. 2). Firstly, uptake is strongly inhibited at concentrations which produces little acceleration of efflux. Secondly,

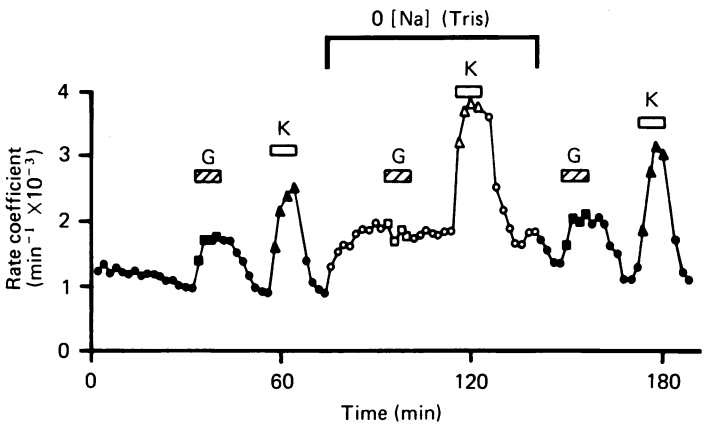

Fig. 5. Effect of inhibiting [^{3}H]GABA re-accumulation from interstitial spaces by substituting 143 mM-[$Tris^+$] for $[Na^+]_o$ on the acceleration of [^{3}H]GABA efflux rate coefficient by 1 mM-[^{3}H]GABA (G) and 140 mM-[K^+] (K, as added KCl).

uptake is inhibited by (at most) half on adding KCl to the incubation medium: of itself, this would increase the rate of efflux by no more than 70 % (see Fig. 13 of Bowery *et al.* 1976). Observed effects of K^+ on efflux are 5–6 times greater than this.

(*b*) Re-uptake can be prevented by adding a carrier-saturating concentration of GABA to the external medium or by totally removing external Na^+ ions: the measured efflux rate is then accelerated 2–3 times (Bowery *et al.* 1976). However, K^+ ions added in the presence of unlabelled GABA (Fig. 1) or in the absence of external Na^+ (Fig. 5), were still capable of acclerating efflux. The effect of K^+ was ; essentially *additive* to uptake inhibition, that is, it produced the same *increment* in rate coefficient but superimposed upon a higher base line (Fig. 6). Conversely, the effect of uptake inhibition was additive with that of raised $[K^+]_o$. In contrast, the effects of Na^+-removal and GABA were mutually *occlusive* (it may also be noted in Figs. 1 and 5 that the acceleration produced by K^+ ions exceeded that resulting from uptake inhibition).

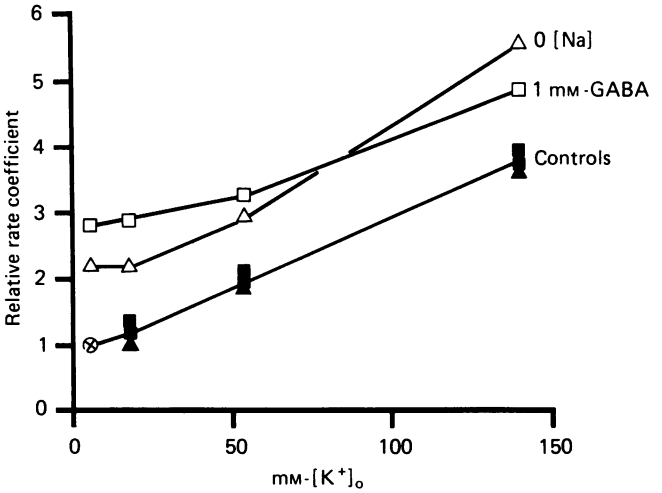

Fig. 6. Relationship between [^{3}H]GABA efflux rate coefficient and external K^+ concentration measured in normal Krebs solution (■, ▲), 0 [Na^+] ($Tris^+$-substituted) solution (△) and Krebs solution containing 1 mM-GABA (□). Rate coefficients are expressed as a fraction of those in normal Krebs solutions ($[K^+]_o$ = 6 mM); triangles and squares refer to two separate experiments.

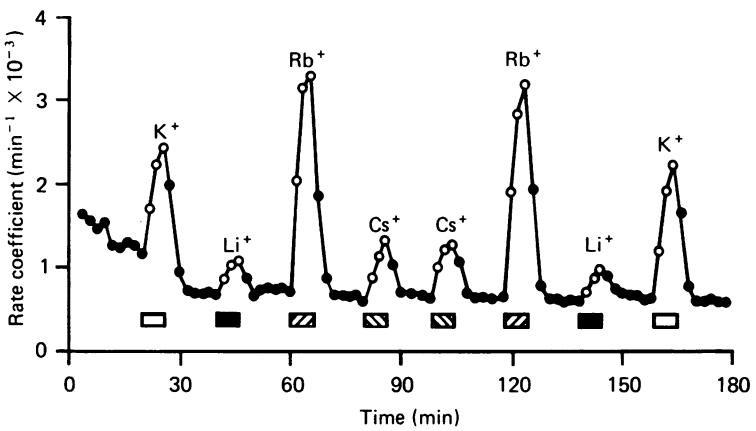

Fig. 7. Effect of different alkali metal cations on [^{3}H]GABA efflux. Cations were added as Cl^- salts (124 mM) in replacement for NaCl to K^+-free Krebs solution (rendered Na^+, 25 mM). The normal superfusion medium was Krebs solution containing 6 mM-(K^+] (4·7 mM-KCl, 1·2 mM-KH_2PO_4); omission of K^+ alone did not change the efflux rate. □, K^+; ■, Li^+; ▨, Rb^+; ▧, Cs^+.

Other alkali metal cations

The effectiveness of other ions (substituted as Cl^- salts for NaCl (124 mM) in a KCl-free solution) as [^{3}H]GABA-releasers diminished in the order $Rb^+ > K^+ > Cs^+ > Li^+$ (Fig. 7).

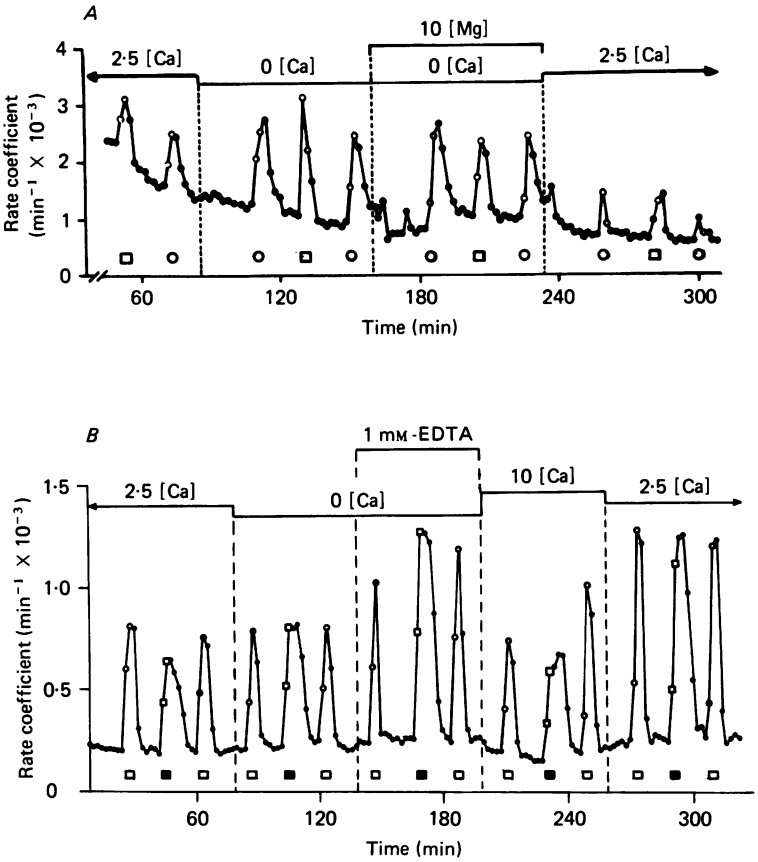

Fig. 8. Effect of (A) Ca^{2+}-free and Ca^{2+}-free/10 mM-$[Mg^{2+}]$ solutions, and (B) Ca^{2+}-free and Ca^{2}-free/1 mM-EDTA solutions, on the efflux of [^{3}H]GABA from two ganglia evoked by 140 mM-$[K^+]$ (□), 1 mM-GABA (■) and electrical stimuli applied across the ganglion body (○; 2 min, 1 Hz, 1 msec, 40 V).

Calcium ions

Omission of Ca^{2+} ions from the external medium, with or without addition of Mg^{2+} (10–20 mM), did not greatly alter either spontaneous or K^+-accelerated [^{3}H]GABA release (Fig. 8*A*), nine experiments). Addition of the chelating agent ethylenediamine tetra-acetate (EDTA, 1 mM) to Ca^{2+}-free solution *increased* both spontaneous and K^+- or GABA-evoked release (Fig. 8*B*; three experiments).

Fig. 9. Effect of 'Ca^{2+}-ionophores' A23187 (*A*) and X537-A (*B*) and (*C*) on [^{3}H]GABA efflux. In *A*, A23187 added during superfusion with Ca^{2+}-free Krebs solution had no effect; subsequent replacement of Ca^{2+} then accelerated efflux. In *B*, addition of X537-A in Ca^{2+}-free solution *did* accelerate efflux; this was further accelerated on replacing Ca^{2+}. In *C* X537-A was first added in a Na^+-free, Ca^{2+}-free solution, and Ca^{2+} (2·5 mM) then added to the Na^+-free solution. (The ionophores were dissolved in methanol (A23187) and acetone (X537-A), then diluted. The diluted solvents had negligible effect, as shown in *A* and *B*.)

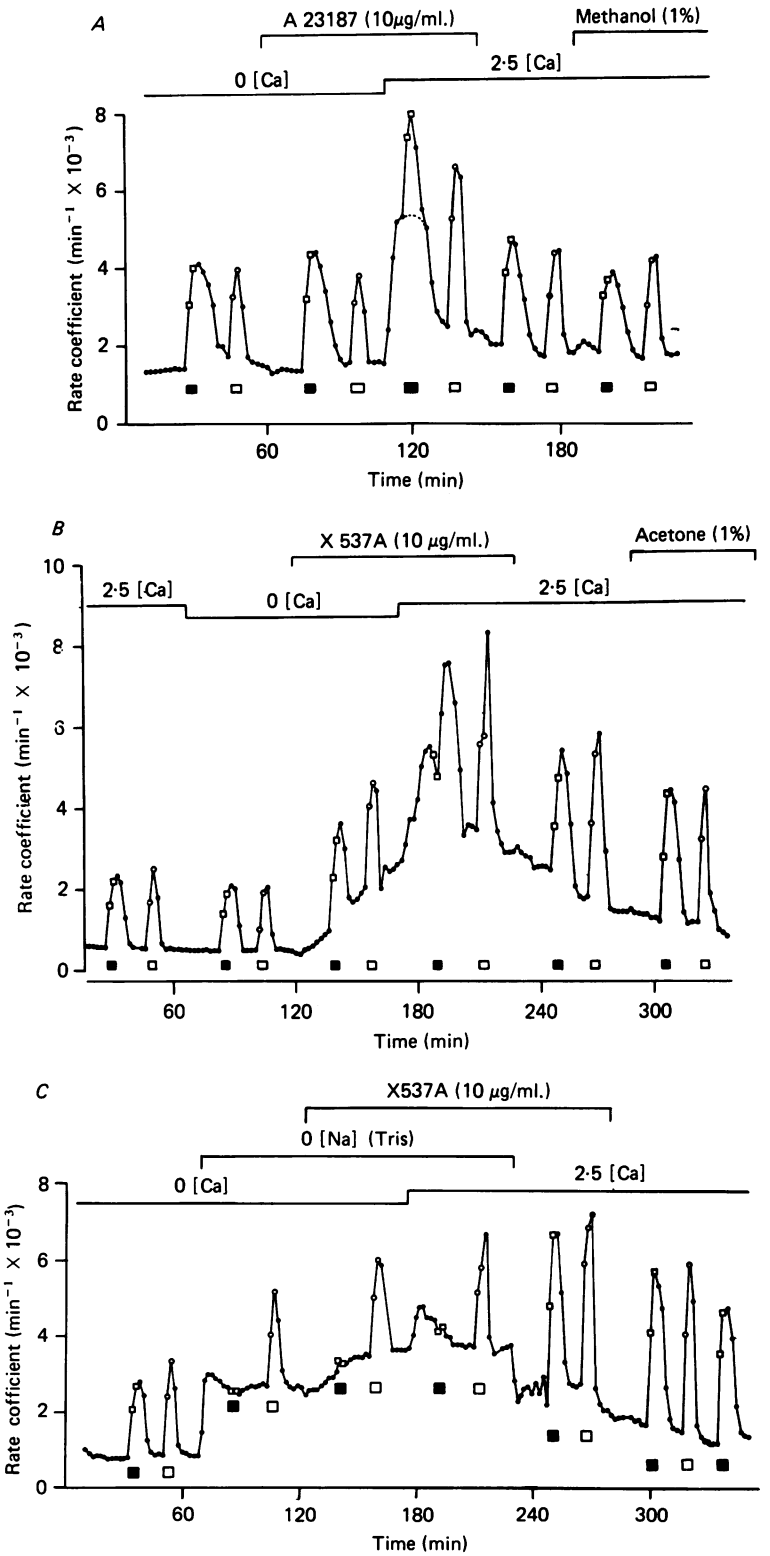
A
A 23187 (10μg/ml.)
Methanol (1%)
0 [Ca]
2·5 [Ca]
Rate coefficient (min⁻¹ X 10⁻³)
Time (min)
B
X 537A (10 μg/ml.)
Acetone (1%)
2·5 [Ca]
0 [Ca]
2·5 [Ca]
Rate coefficient (min⁻¹ X 10⁻³)
Time (min)
C
X537A (10 μg/ml.)
0 [Na] (Tris)
0 [Ca]
2·5 [Ca]
Rate cofficient (min⁻¹ X 10⁻³)
Time (min)

Fig. 9.

Addition of the Ca^{2+}-ionophore A23187 (Pfeiffer, Reed & Lardy, 1974) did not alter the rate of efflux of [^{3}H]GABA in the absence of external Ca^{2+}, but subsequent replacement of Ca^{2+} (with A23187 still present) produced a transient acceleration of release (Fig. 9). The effects of X537-A (Pressman, 1973) were more complex, in that a substantial acceleration of [^{3}H]GABA release did occur in the absence of Ca^{2+}, which was further increased by replacing Ca^{2+} (Fig. 9). The initial effect in Ca^{2+}-free solution might have resulted (in part) from Na^+-entry (Devore & Nastuk, 1975; Cochrane & Douglas, 1975) since it was smaller in a $Tris^+$-substituted Na^+-free

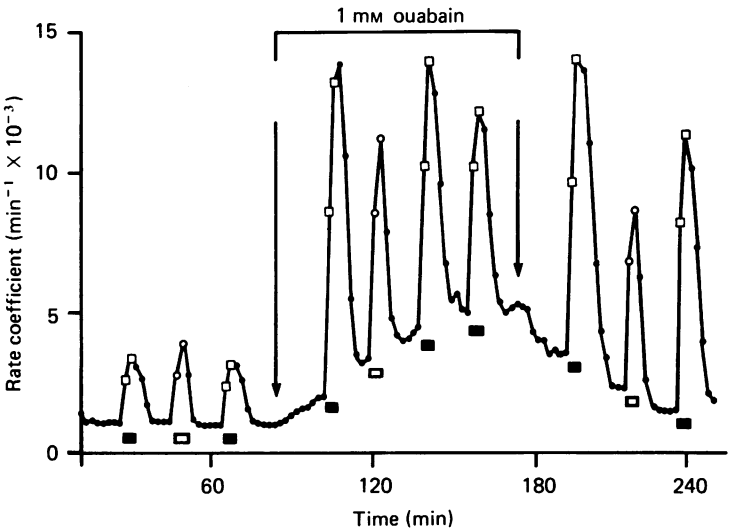

Fig. 10. Effect of 1 mM-ouabain on the rate coefficient for [^{3}H]GABA efflux. 1 mM-GABA (■) and 140 mM-[K^+] (□, as KCl) were added for 4 min.

solution (Fig. 9). Under these conditions, replacement of Ca^{2+} then produced a transient further acceleration, like that produced by A23187. Both agents had rather more pronounced effects on the release of tritium from ganglia preloaded with [^{3}H]choline (see below).

Sodium ions

An attempt was made to raise *intracellular* Na^+ using ouabain (see Brown & Scholfield, 1974*a*; Scholfield, 1975). This produced a gradual rise in the spontaneous efflux rate accompanied by a marked potentiation of the responses to GABA and K^+ ions (Fig. 10). The fractional increment in the ratio of evoked/resting efflux rates remained approximately the same during ouabain application as before, but the evoked release remained enhanced after spontaneous efflux had recovered. The time course of the effect of ouabain on spontaneous release accords with a slow accumulation of intracellular Na^+ ions (Scholfield, 1975 and unpublished experiments), but whether this or the accompanying depolarization is responsible for the acceleration remains uncertain.

Electrical stimulation

Characteristic effects of electrical 'stimulation' of the ganglion on the release of [^{3}H]GABA from a superfused preparation are illustrated in Fig. 11. Application of electrical pulses across the *soma* of the ganglion produced a sharp acceleration of efflux which tended to diminish appreciably with repeated tests. Application of comparable pulses to the *preganglionic nerve trunk* produced a much smaller release, again declining with repeated pulses. Responses to raised $[K^+]_o$ interposed between

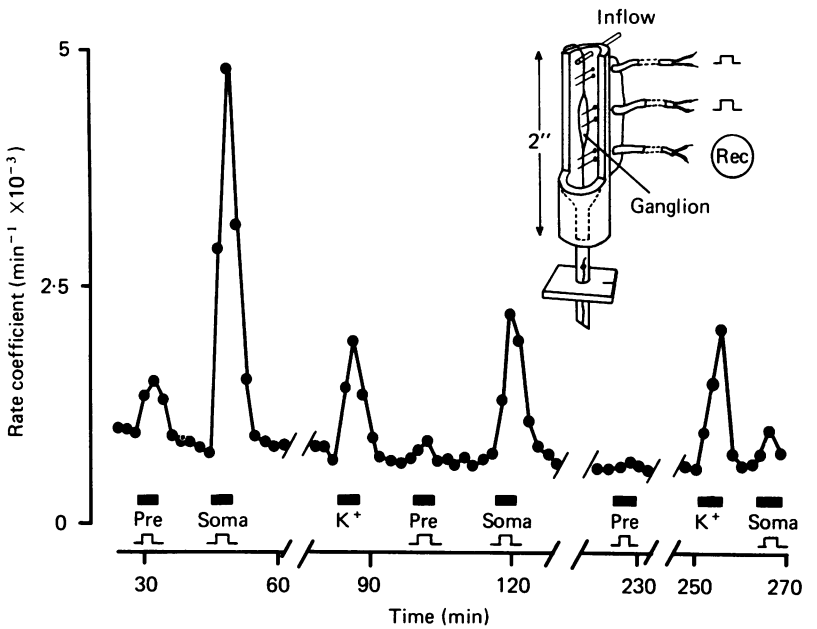

Fig. 11. Effect of applying 4 min trains of electrical pulses (⎍, 1 Hz, 0·5 msec duration, 10 V) to the preganglionic nerve trunk ('Pre') and to the body of the ganglion ('Soma') on the rate coefficient for [^{3}H]GABA efflux from a superfused rat ganglion preparation. The diagram above shows the arrangement for stimulating (⎍) the two parts of the preparation, using bipolar platinum electrodes, and for recording the post-ganglionic response ('Rec'). The stimulus parameters evoked post-ganglionic action potentials. Note that the responses to electrical stimulation waned whereas those to 140 mM-[K+] (as KCl, 'K^+') did not.

periods of electrical stimulation did not decline. Electrical stimulation increased the efflux rate of [^{14}C]urea by no more than 30%, i.e. much less than that of [^{3}H]GABA.

The diminishing response to repeated stimuli precluded any systematic analysis of the effects of varying the release parameters. In general, minimally effective stimuli were pulses of 0·1 msec duration of 10–40 V delivered at a frequency of 1 Hz in 4 min trains. Increasing the stimulus duration (to 2 msec) or frequency (to 100 Hz) increased the amount of [^{3}H]GABA released without any clear ceiling effect.

Since the minimal stimulus parameters required to release [^{3}H]GABA were comparable to those required to excite nerve fibres in the ganglion – judged from post-ganglionic action potential generation – release might have resulted in part as an indirect consequence of neural activation. Two types of experiment were undertaken

to test this: the use of blocking agents against nerve excitation or transmission, and the use of a partitioned bath (see Methods) to test for propagation.

(*a*) *Blocking agents*

Somatic stimulation. The local anaesthetics amethocaine (33–300 μM) and procaine (3·7 mM), applied in concentrations which blocked fibre conduction, did *not* prevent the effects of electrical pulses applied to the ganglion soma (Fig. 12): if anything,

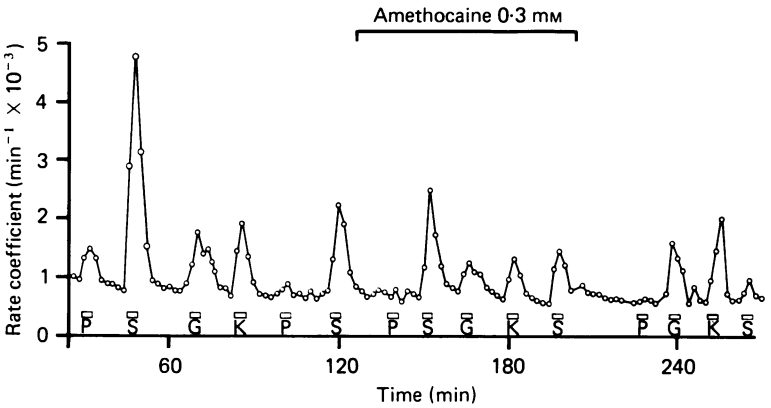

Fig. 12. Effect of 0·3 mM-amethocaine on the acceleration of [^{3}H]GABA efflux from a superfused ganglion produced by 4 min periods of preganglionic nerve stimulation (P), stimulation of the ganglion soma (S), addition of 152 mM-KCl (K) or addition of 1 mM-GABA (G). The electrical stimuli (0·5 msec, 1 Hz, 30 V) evoked post-ganglionic nerve action potentials, which were blocked in the presence of amethocaine.

responses appeared greater. However, they did reduce the response to GABA or to raised external [K^+], presumably by reducing the turnover rate of the carrier. Pentobarbitone (1 mM) had similar effects. The response to somatic stimulation was also resistant to Ca^{2+}-free high [Mg^{2+}] solution (Fig. 8*A*) and to 2·5 mM-hexamethonium, indicating that presynaptic terminal excitation was not involved.

Preganglionic stimulation. The type of 'internally controlled' experiment illustrated in Fig. 12 was inapplicable to release evoked by preganglionic nerve stimulation, since the amount of radioactivity released was smaller and declined to undetectable levels after two or three periods of stimulation. Instead, the effects of blocking agents on responses to different preparations to a single period of stimulation were compared. Results are summarized in Table 3. In essence, none of the blocking agents used – amethocaine, tetrodotoxin or Ca^{2+}-free, high-Mg^{2+} solution – depressed the amount of [^{3}H]GABA released: on the contrary, release seemed to be enhanced.

(*b*) *Propagation*

In these experiments the preparation was mounted in a partitioned bath to allow the separate collection of radio-activity both from the region to which stimuli were applied and also from more remote regions (see Bowery & Tulett, 1975). In consequence it became possible to find out if (for example) the [^{3}H]GABA released by preganglionic nerve stimulation derived solely from the preganglionic trunk or from

TABLE 3. Fractional increment (peak/basal) in [^{3}H]GABA release from superfused rat ganglia produced by a 4 min train of electrical pulses (2 Hz, 1 msec duration) applied to the preganglionic nerve trunk. In each experiment the stimulus voltage was adjusted to produce a maximal post-ganglionic action potential at the beginning of the experiment: the procedures used (excepting controls) all blocked this response.

	Individual measurements			Mean
Controls	2·68	9·83	1·17	4·56
Amethocaine (300 μM)	17·47	7·02	11·77	12·09
Tetrodotoxin (3 μM)	2·45	16·83	9·02	9·43
0 mM-[Ca^{2+}]/30 mM-[Mg^{2+}]	3·86	13·69	23·50	13·68

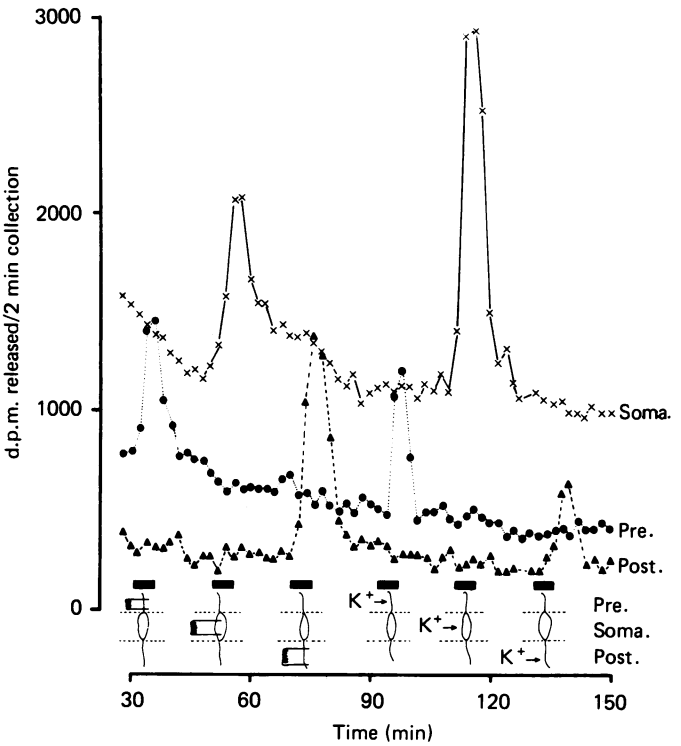

Fig. 13. A test for propagation of responses to electrical stimulation. The ganglion preparation was arranged horizontally in a 3-chambered bath such that pre- and post-ganglionic nerve trunks and ganglion soma could be individually stimulated by electrical pulses (1 msec, 10 Hz) or by adding 140 mM-KCl, and the efflux of [^{3}H]GABA from the stimulated region and from the other two non-stimulated regions measured separately (see Bowery & Tulett, 1975). Points show the *amount* of [^{3}H]GABA released (in d.p.m. per 2 min collection period) from the soma (×), preganglionic trunk (●) and post-ganglionic trunk (▲); the region stimulated is indicated below the curves. Each stimulus released [^{3}H]GABA *only* from the stimulated region, even though preganglionic stimulation evoked a post-ganglionic action potential.

propagation of the stimulus to the ganglion soma. An illustration of this is shown in Fig. 13. Wherever the stimulus was applied – preganglionic trunk, soma or post-ganglionic trunk – [^{3}H]GABA was only released from the stimulated region, i.e. there was no propagation of the stimulus from one region to the next. This was confirmed in several preparations, including a segment of vagus nerve trunk (which

also takes up GABA; see Bowery & Brown, 1972). In contrast, there *was* detectable release of tritium from the somatic compartment on stimulating the preganglionic trunk when the ganglion had been prelabelled with [^{3}H]choline (Fig. 14). This probably reflected release of synthesized [^{3}H]acetylcholine from the nerve terminals (although hydrolysed back to [^{3}H]choline in the absence of cholinesterase; see Perry 1953; Collier, 1969; Brown, Jones, Halliwell & Quilliam, 1970) since release following preganglionic nerve stimulation was blocked in a Ca^{2+}-free, high-Mg^{2+} solution (Fig. 14).

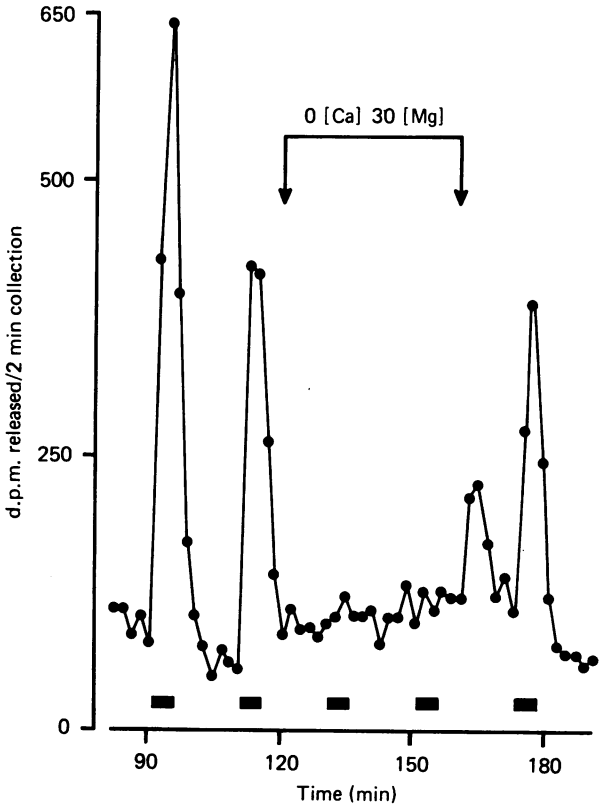

Fig. 14. Amounts of tritium released from the *soma* of a superior cervical ganglion preloaded with [^{3}H]choline on stimulating the *preganglionic trunk* for 4 min at 30 Hz (1 msec pulses, of sufficient voltage to generate a post-ganglionic action potential). In this experiment the ganglion was mounted in a 3-chambered bath (as in Fig. 13) so that efflux of tritium from the somatic region only was monitored. Stimulated efflux was suppressed by perfusing the somatic region with a Ca^{2+}-free solution containing 30 mM-[Mg^{2+}], which blocked transmission.

Release of [^{3}H]leucine

Low concentrations of [^{3}H]L-leucine ($< 1\ \mu$M) are taken up primarily by the *neurones* in sympathetic ganglia, in contrast to the glial cell target for GABA (Bowery *et al.* 1979; see also Schon & Kelly, 1974). Although most of the ^{3}H retained in the ganglia appears to be in the form of metabolites, the fact that the radioactivity (whatever its nature) is concentrated in the neurones offered an interesting possibility of enquiring how the release of labelled material from the neurones compared

with release of [³]GABA from glial cells. With this aim, efflux of tritium from isolated ganglia pre-incubated in [³H]L-leucine (0·24 μM) for 2–3 hr was measured in seven ganglia. A representative experiment is illustrated in Fig. 15. Essential observations were as follows.

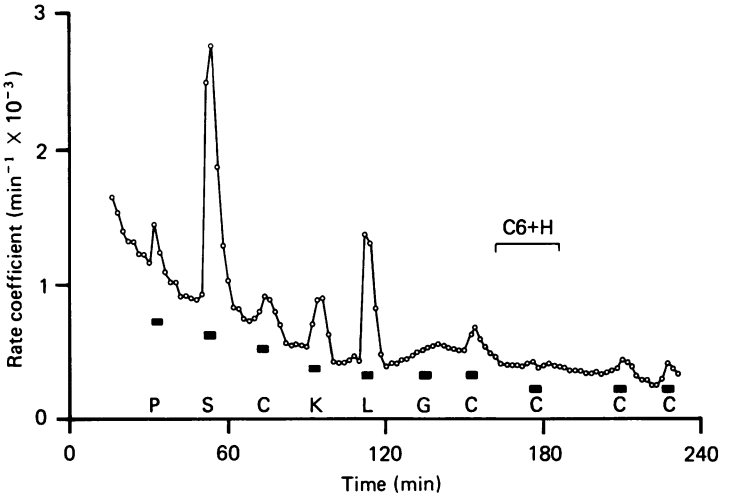

Fig. 15. Efflux of tritium from a ganglion pre-incubated for 3 hr with 0·24 μM-[³H]L-leucine. Stimuli as follows were applied for 4 min periods: P and S preganglionic nerve and somatic electrical stimulation at 10 Hz, 1 msec; K, perfusion with 140 mM-[K⁺] (as added KCl); C, addition of 550 μM-carbachol; G, addition of 1 mM-GABA; L, addition of 1 mM-L-leucine. The response to carbachol was blocked by addition of 2·5 mM-hexamethonium (C6) plus 1·4 μM-hyoscine (H).

(*a*) The basal rate coefficient for tritium efflux diminished slowly over 3–4 hr superfusion to a minimum value between 0·3 and 0·6 × 10^{-3} min^{-1}, quite similar to that for [³H]GABA release.

(*b*) Tritium efflux was not affected by adding 1 mM-GABA to the superfusion fluid but *was* accelerated 3–4 times by 1 mM-L-leucine. This suggests that a large part of the effluent tritium does refer to unmetabolized [³H]leucine ([³H]leucine uptake is inhibited by unlabelled leucine but not by GABA: Bowery *et al.* 1979).

(*c*) Addition of 120–150 mM-KCl accelerated efflux by 1·5–2 times, rather less than the corresponding acceleration of [³H]GABA release.

(*d*) Efflux was consistently accelerated by *carbachol*: this effect was prevented by adding hexamethonium and hyoscine in concentrations (2·5 mM and 1·4 μM respectively) sufficient to annul ganglion depolarization (carbachol did *not* modify [³H]-GABA release: Bowery & Brown, 1972).

(*e*) Efflux was also accelerated by electrical stimulation of the ganglion soma or preganglionic nerve trunk.

Qualitatively similar effects of K^+, unlabelled leucine and carbachol were observed in ganglia preloaded and superfused in the presence of cycloheximide (20 μg/ml.), to prevent incorporation of [³H]leucine into protein (see Bowery *et al.* 1979). The major effect of cycloheximide was to increase the resting rate coefficient for release to about 10^{-2} min^{-1}, presumably because a greater fraction of accumulated [³H]leucine was now releasable.

DISCUSSION

There were two main aims to these experiments. *First*, our original observation (Bowery & Brown, 1972) that high concentrations of K^+ ions or electrical currents could accelerate the release of accumulated [^{3}H]GABA from isolated ganglia seemed anomalous, since these were stimuli normally used to discharge suspected transmitter substances from nerve endings; yet GABA is taken up by (and presumably released from) *glial* cells in sympathetic ganglia, not nerve endings (Young *et al.* 1973). We wished, therefore, to find out how the release of GABA from the ganglion resembled or differed from that of a true neurotransmitter. *Secondly*, with a sufficiently fast release from the glial cells neighbouring neurones in the ganglion are depolarized by the GABA (Bowery *et al.* 1976). We wondered whether K^+ ions might provide a suitable 'physiological' stimulus for such a 'neuromodulatory' release, and whether the previously observed effect of electrical current might imitate a physiological release process. In the latter case, we envisaged the possibility that electrical stimulation might be releasing GABA, not through a direct action on the glial cells, but instead by exciting neurones, with the consequential rise in interstitial K^+ acting as the final trigger for release.

The present experiments show that the release of GABA from ganglionic glial cells can be differentiated from an exocytotic transmitter release process; that such release as we have observed is unlikely to be of physiological importance; and that the effects of electrical currents are not mediated through excitation of neurones or nerve fibres, but instead are due to a direct, possibly traumatic, action on the glial cells.

Comparison with transmitter release

K^+-evoked release. This differed from that expected for neurotransmitter release from nerve endings in two important respects. (i) GABA efflux-rates from sympathetic ganglia were relatively insensitive to changes in external [K^+] below 50 mM and, above 70 mM-$[K]_o$, increased with the first power of K^+ concentration. In contrast, transmitter release at, for example, the neuromuscular junction is markedly accelerated by much smaller increments in $[K^+]_o$, and, in the presence of a Ca^{2+}/Mg^{2+} ratio similar to that which we have used, increases as the fourth power of $[K^+]_o$ above 10 mM (Liley, 1956). The relative insensitivity of GABA-release to K^+ ions cannot be attributed to a defective response of the glial cell membrane potential, since the membrane potential of impaled (presumed) glial cells shows a reasonable Nernst-relationship to $[K^+]_o$ with a slope of 49 mV/decade concentration change down to 2 mM (see Appendix). Although physical clearance from the intact ganglion might set a ceiling to the overflow of GABA at high efflux rates, clearance restrictions would not account for the insensitivity below 50 mM-$[K^+]_o$. A very appropriate comparison is provided by the overflow of radiolabelled acetylcholine from isolated rat ganglia: this is accelerated a hundredfold at 40 mM $[K^+]_o$ (A. J. Higgins, personal communication). (ii) The K^+-evoked acceleration of GABA release was *not* 'Ca^{2+}-dependent'. On the contrary, the effects of K^+ ions were *augmented* in a Ca^{2+}-free solution. In contrast,the evoked release of radiolabelled acetylcholine from the nerve endings was rapidly suppressed in a Ca^{2+}-free, high Mg^{2+} solution (Fig. 14 and

unpublished observations). Likewise, the release of GABA from synaptosomal nerve endings is prevented by removing Ca^{2+} ions (e.g. De Belleroche & Bradford, 1972; Levy, Haycock & Cotman, 1974; Redburn, Shelton & Cotman, 1976). Since there is no evidence for exocytotic release from a vesicular store of the GABA in the ganglionic glial cells this insensitivity to Ca^{2+} ions is not surprising.

GABA is also selectively taken up by glial cells in sensory ganglia (Schon & Kelly, 1974). In this preparation Minchin & Iversen (1974) found detectable acceleration of [^{3}H]GABA release down to 20 mM $[K^+]_0$, and further reported that such release *was* inhibited in a Ca^{2+}-free + EDTA solution (see also Roberts, 1974). The reason for this difference between sensory and sympathetic ganglia is not fully clear. One factor might be that part of the release reported by Minchin & Iversen (1974) and Roberts (1974) appears to have stemmed from an effect on the re-uptake of effluent GABA from the interstitial spaces, since the action of K^+ ions was inhibited in a Na^+-free medium (Minchin, 1975): in the present experiments the action of K^+, albeit, at higher concentrations, persisted in a Na^+-free solution or in a solution where re-uptake was inhibited by carrier-saturating concentrations of GABA (Figs. 5 and 6). It may be noted that the K^+ evoked release of β-alanine (also from glial cells: see Schon & Kelly, 1975; Bowery *et al.* 1979) from the frog spinal cord (Adair & Davidson, 1977) and rat cerebral cortex (Johnston, 1977) is not sensitive to Ca^{2+} ions.

The *mechanism* whereby high concentrations of K^+ accelerate the release of GABA from the sympathetic ganglion remains unclear. It does not appear to result from osmotic changes, nor, as indicated above, from an increased overflow following inhibition of carrier-mediated re-accumulation. The obvious explanation that it results from membrane depolarization is supported by the order of cation effectiveness, but there remain the difficulties of explaining why a depolarization of probably 40–50 mV is necessary to increase the efflux rate (see Appendix), and why there is such a marked asymmetry in the effects of K^+ ions on uptake and release (see Fig. 2).

Blaustein & King (1976) have analysed the K^+ dependence of GABA *uptake* into synaptosomal nerve endings in terms of a Na^+-coupled, carrier-mediated transport system, such that, if the Na^+-carrier-GABA complex is positively charged, the rate coefficient for transport might show an exponential dependence upon transmembrane voltage of the form

$$k = k(0)\exp(n\,FV/RT) \tag{1}$$

where $k(0)$ is the rate coefficient at zero membrane potential ($V = 0$), and n is the valency of the transporting complex. Then, if $V = RT/F \ln([K^+]_o/[K^+]_i)$,

$$k \propto k(0)\,[K^+]_o{}^n. \tag{2}$$

Synaptosomal uptake accorded with eqn. (2), but with a power-function less than unity: Blaustein & King (1976) interpreted this as being compatible with a valency of 2 for the transporting complex (see also Martin, 1976) if the complex only 'sees' a fraction (0·2) of the transmembrane potential. Above 70 mM-$[K^+]_o$ the present observations on GABA *efflux* are also compatible with eqn. (2), with $n = 1$. However, although the most plausible mechanism, there is no direct evidence that GABA exit in the ganglion is carrier-mediated: no clear saturation was detectable up to internal GABA concentrations of 1 mM (in contrast to influx, which saturates with an apparent K_T of 7 μM: Bowery *et al.* 1979); no exchange-diffusion has been unequivocally demonstrated (Bowery *et al.* 1976, 1979); and though acceleration of exit by ouabain might indicate a Na^+-coupled process, other explanations are possible.

Electrical stimulation. The release of GABA from the ganglion by current pulses also differs from a transmitter-release process in its insensitivity to Ca^{2+} ions. The current seemed to exert a direct and localized effect on the glial cells, which was not

secondary to excitation of neurones or nerve fibres since the response was not reduced by local anaesthetics or tetrodotoxin. The decremental pattern of release recalls the observations of Dennis & Miledi (1974) on the release of acetylcholine from Schwann cells at denervated motor end-plates by focal currents: this was accompanied by visible signs of cell damage, and was attributed to a break-down of the cell membrane. A comparable 'traumatic' release might well explain the present observations.

Nevertheless, even if artifactual, the effect of electrical currents on ganglia has some *practical* importance in connexion with the use of this type of 'field' stimulation to release GABA as a suspected transmitter substance from isolated or homogenized nervous tissue (see, for example, Srinivasan, Neal & Mitchell, 1969; Katz, Chase & Kopin, 1969; Bradford, 1970; Hammerstad, Murray & Cutler, 1971; De Belleroche & Bradford, 1972). In such experiments transmitter release would need to be distinguished very clearly from the type of artifactual release we have observed. It should also be noted that such artifactual release is not confined to substances in glial cells, since radiolabelled leucine was also released (probably from the ganglionic neurones: Bowery *et al.* 1979). The use of urea as a detector for 'non-specific' release (cf. Srinivasan *et al.* 1969) does not seem particularly helpful, since electrical stimulation did not accelerate urea release from ganglia. Another neutral amino acid may be better (Orrego, Jankelevich, Ceruti & Ferrera, 1974) but reciprocal sensitivity to Ca^{2+} and Mg^{2+} ions remains the clearest method of discriminating true transmitter release.

Numerically, the rate of release would need to be increased by about 2–3 times for even minimal effects on adjacent neurones in the ganglion (Bowery *et al.* 1976). This would require an interstitial K^+ concentration of nearly 100 mM (Fig. 2*A*), an order of magnitude greater than the maximum interstitial levels achieved during repetitive nerve activity (10–12 mM, see Appendix). Indeed, the range of K^+ concentrations necessary to stimulate GABA release, even if attained locally, would of themselves block nerve conduction, so rendering any 'modulatory' effect of GABA superfluous. Unless the release process were some 10 times more sensitive to K^+, or some other substance were to act as the stimulus for release, the same conclusion seems inevitable at other sites.

In conclusion, the efflux of GABA from the glial cells may best be regarded as simply an obligatory 'leak' consequential upon the generation of high intracellular concentrations by the carrier-mediated uptake process. As such, its 'function' resides in the influence it has on the ability of the uptake process to hold down the interstitial GABA concentrations (Bowery *et al.* 1979). Only under pathological conditions where, for example, the intraglial concentration of free GABA is raised by inhibiting transamination or uptake is inhibited by pharmacological agents (Bowery *et al.* 1976) might the exit process provide an effective source of GABA.

This investigation was aided by a grant from the Medical Research Council. A number of the experiments described above, and in the Appendix, were performed in the Department of Pharmacology at St Bartholomew's Hospital Medical College: we thank Professor J. P. Quilliam for his hospitality.

APPENDIX

By P. R. Adams and D. A. Brown

Electrical responses of presumed sympathetic neuroglial cells to K^+ ions and to preganglionic nerve stimulation

During the course of previously described experiments on neurones in isolated rat superior cervical ganglia (Adams & Brown, 1975), a number of 'electrically silent' cells were impaled. Although not positively identified by, for example, dye-marking, the cells were presumed to be neuroglial cells since their electrical properties closely matched those described for other neuroglial cells in both invertebrate (Kuffler & Nicholls, 1966) and vertebrate (Kuffler *et al.* 1966; Dennis & Gerschenfeld, 1969; Somjen, 1975) nervous systems. Blackman, Crowcroft, Devine, Holman & Yonemura (1969) and Blackman & Purves (1969) have described similar cells in guinea-pig ganglia, and have drawn the same conclusion.

The cells in question showed: (i) a higher initial resting potential than is usual for sympathetic neurones (up to -90 mV) immediately on impalement, as against -70 mV at most for neurones (cf. Perri, Sacchi & Casella, 1970; Adams & Brown 1975); a low input resistance ($\leqslant 10$ MΩ) and short time constant (< 5msec); (ii) no spike following the injection of several nA of depolarizing current through the micro-electrode; and (iii) no synaptic potential, but instead a characteristic 'slow potential' (see below) following orthodromic (preganglionic) nerve stimulation. Sometimes (either spontaneously or through a very small micromanipulator advance) the micro-electrode appeared to slip directly from such a cell into a neurone indicated by a sudden fall in membrane potential of 20–30 mV accompanied by an *increased* input resistance, and the appearance of the usual excitable responses. It seems probable that the first cell impaled was the satellite cell surrounding the neurone; other presumed-glial cells might have been satellite cells or Schwann cells surrounding the intraganglionic fibres and cell process.

Since these cells might have been representative of those responsible for the accumulation and release of [^{3}H]GABA described in the present and preceding paper (see Young *et al.* 1973), it seemed worthwhile checking their electrical responses to K^+ ions and to orthodromic nerve stimuli, two of the stimuli found to release [^{3}H]GABA. The membrane potential of most of the numerous cells impaled dissipated very rapidly (within a minute or so) but occasionally settled to a fairly steady level of between -65 and -85 mV. The observations described below were made on eight such cells.

Effect of K^+ ions

Relatively small increases in $[K^+]_0$ from the normal level of 5·9 to 12, 18 or 24 mM produced rapid, sustained and reversible depolarizations (Fig. 16); conversely, reduction to 2 mM hyperpolarized the cells. Fig. 16 shows the relationship between membrane potential and $[K^+]_0$ (over a limited range) in two cells from different ganglia which had similar stable resting potentials. The slope of the linear portion

of the curve is 49 mV per decade change in $[K^+]_o$ and extrapolates to 200 mM at zero membrane potential. This latter value accords with total intracellular $[K^+]$ measured by flame photometry in rat ganglia (Brown & Scholfield, 1974*a*) and with estimates of $[K^+]_i$ in sympathetic glial cells in tissue culture derived from the

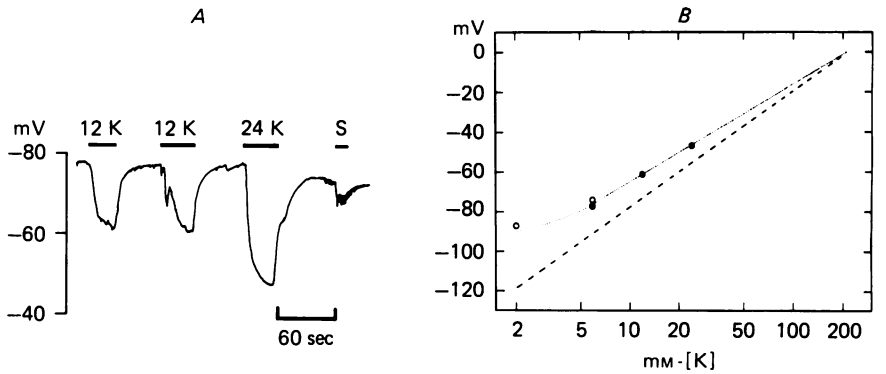

Fig. 16. Responses of presumed glial cells in isolated rat superior cervical ganglia to changes in external (K^+). Cells were impaled with K^+ acetate-filled micro-electrodes (resistance 40–100 MΩ). The ganglion was superfused with Krebs solution at 21–26 °C, at a rate adjusted to give a bath-exchange time of 2–4 sec (see Adams & Brown, 1975, for further details). *A*, DC recording of membrane potential changes produced by raising $[K^+]_o$ from 5·9 to 12 and 24 mM. At S, the preganglionic nerve trunk was stimulated at 6 Hz (see Figs. 2 and 3). *B*, pooled results from this (●) and another (○) cell with similar initial resting potential. The interrupted line (– –) indicated the relationship expected for a perfect K^+-permselective cell (slope 59 mV/decade change in $[K^+]_o$); the dotted line (- - - -) shows a curve drawn according to eqn. (1). The K^+ concentration of the solution was adjusted by removing or adding KCl.

distribution of ^{86}Rb (Brown & Shain, 1977). Assuming $[K^+]_i = 200$ mM the membrane potential could then be described by the expression:

$$E = 59 \log \left[\frac{[K^+]_o + \alpha[Na^+]_o}{[K^+]_i} \right], \quad (1)$$

where $[Na^+]_o = 143$ mM and α $(= P_{Na}/P_K) = 0{\cdot}025$.

This degree of K^+ permselectivity accords quite well with that reported for other mammalian neuroglial cells (Dennis & Gerschenfeld, 1969; Pape & Katzmann, 1972; Ransom & Goldring, 1973*a*), though rather less than that in leech (Kuffler & Nicholls, 1966) or amphibian (Kuffler *et al.* 1966) cells. As pointed out by Dennis & Gerschenfeld (1969), an imperfect Nernst response to K^+ might result in part from imperfect micro-electrode sealing. This possibility is supported by the very high value of the resting potentials obtained immediately on impalement, which often approached that (−90 mV) estimated for E_K on the basis of measured cation concentration gradients (Brown & Scholfield, 1974*a*; Brown & Shain, 1977; see also Fig. 16). Irrespective of the precise degree of K^+ permselectivity, it is clear from these experiments that the failure of alterations in external K^+ concentration below 50 mM to change the rate of

[³H]GABA efflux (Fig. 2) cannot be attributed to any peculiar insensitivity of sympathetic glial cell membrane potential to K^+ ions.

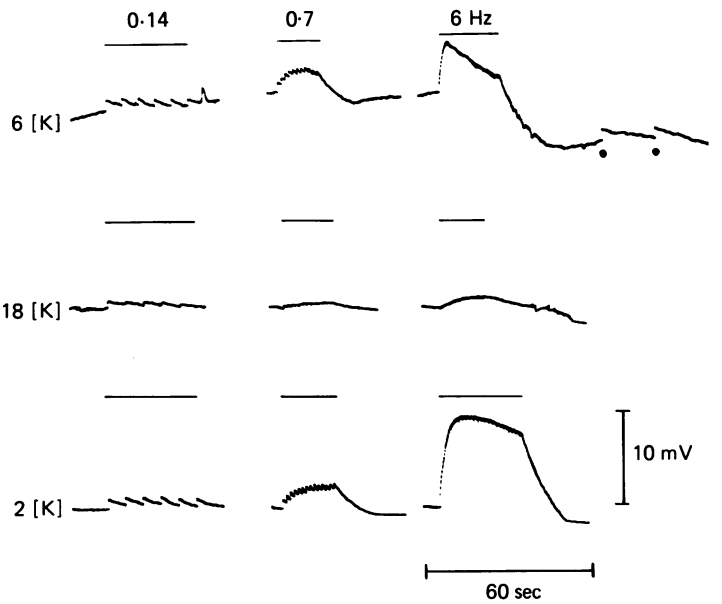

Fig. 17. Changes in membrane potential of a presumed glial cell accompanying orthodromic (preganglionic) nerve stimulation at three different frequencies (0·14, 0·7 and 6 Hz) observed in solutions containing 6, 18 and 2 mM-[K^+] (depolarization *upwards*, cf. Fig. 16). The stimulus parameters were such as to evoke transmitted action potentials in a nearby neurone in the same ganglion: these showed intermittent failure in 18 mM-[K^+] solution. During the hyperpolarization ensuing after 6 Hz stimulation in 6[K^+], two orthodromic stimuli were delivered (at the dots). Hyperpolarization also followed 6 Hz stimulation at 2[K^+] but is attenuated by recorder saturation.

Effects of preganglionic nerve stimulation

Single orthodromic stimuli applied to the preganglionic nerve trunk (of sufficient intensity to evoke a transmitted action potential from the neurones) produced transient depolarizing deflexions of between 1·1 and 1·4 mV (Fig. 17). These decayed much more slowly than synaptic potentials, with a time constant of around 10 sec. Assuming these deflexions to reflect fluctuations in interstitial [K^+] (Orkand, Nicholls & Kuffler, 1966) and, using Fig. 16*B* as a calibration curve, the deflexions would correspond to increments of 0·3–0·4 mM in periglial [K^+] with each nerve impulse.

During *repetitive* preganglionic stimulation the depolarization incremented to a maximum value (at 3–6 Hz) of between 5 and 10 mV, corresponding to a maximum increment of 2–4·5 mM periglial [K^+].

Reduction of $[K^+]_o$ from 6 to 2 mM enhanced the depolarizing responses to repetitive orthodromic stimulation, as expected if the depolarization were due to a fixed *increment* in periglial [K^+]. Responses were strongly depressed on raising

$[K^+]_o$ to 18 mM: in part this might have resulted from an irregular failure of orthodromic transmission, apparent on recording from neurones.

During prolonged trains of orthodromic stimuli the depolarizing response waned somewhat, and then reversed to a clear *hyper*polarization on stopping the stimulation. Fig. 17 shows an example of this: it also shows that the response to a single stimulus applied during this hyperpolarizing phase was somewhat augmented.

The estimated increment of 0·3–0·4 mM/impulse in ganglia is fairly comparable with that (0·23–0·26 mM) measured by Orkand *et al.* (1966) in amphibian optic nerves, but rather less than that (0·92 mM) estimated for cat cerebral cortex (Ransom & Goldring, 1973*b*). This might reflect differences in the dimensions of the interstitial spaces. The peak depolarization obtained during repetitive orthodromic stimulation (5–10 mV) was less than that previously reported for guinea-pig ganglia by Blackman & Purves (1969). In part this may reflect the lower maximum discharge frequency of the rat ganglion cells under the present experimental conditions (< 10 Hz). A second factor is the waning depolarization observed during sustained stimulation, reversing to a hyperpolarization on stopping the stimulus. This has been noted before in cortical glial cells (Ransom & Goldring, 1973*c*), and attributed to a reduction of interstitial $[K^+]$ consequent upon active removal: this would tend to set a ceiling of the amount of K^+ accumulated.

The calculated peak increments in interstitial $[K^+]$ during repetitive orthodromic stimulation agree reasonably well with those recently reported in this tissue using K^+-sensitive electrodes (2·5–8·5 mM: Friedli, 1978; about 6 mM at 8 Hz: Galvan, Ten Bruggencate & Senekowitsch, 1979). Such increments, giving an over-all concentration of 10–12 mM interstitial $[K^+]$, are clearly below the threshold necessary to accelerate GABA-release (cf. Fig. 2); hence released K^+ is unlikely to serve as an effective physiological stimulus for GABA-release from neuroglial cells.

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