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THE APPEARANCE AND DEVELOPMENT OF CHEMOSENSITIVITY IN ROHON-BEARD NEURONES OF THE *XENOPUS* SPINAL CORD

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SUMMARY

1. We have examined the onset and subsequent development of chemosensitivity in Rohon–Beard neurones from the *Xenopus* spinal cord. These cells become sensitive to bath-applied γ-aminobutyric acid (GABA) around stage 25 (early tailbud, about 1 d old), and remain so at least until stage 49 (9 d old). In contrast, a number of other neurotransmitter candidates tested caused no potential or conductance change during the same period.

2. We examined ionophoretic dose–response relations of the cells at stage 26, a couple of hours after the first acquisition of GABA sensitivity. Sensitivities as high as 450 mV/nC were recorded. Comparable sensitivities were recorded between stages 46–49 (5–9 d old).

3. Measurements of ionophoretic sensitivities and input resistances during several periods from stage 26 to maturity show that the underlying conductance change for a given GABA dose is likely to increase steadily during this time. A 'sensitivity index' (ionophoretic sensitivity/input resistance) was calculated, which is low at stage 26, higher at intermediate stages (stages 31–42), and highest for mature cells (stages 46–49; 5–9 d of development).

4. The reversal potential of the ionophoretic GABA response is the same at stage 26 (−30 mV) as it is in mature cells. Ion substitution experiments show that Na^+ and K^+, but not Cl^- or Ca^{2+}, are involved in the response.

5. GABA responses at stage 26 are pharmacologically similar to those of mature cells. The responses are blocked by 10 μM-picrotoxin or curare, and muscimol is an agonist in concentrations as low as 1 μM.

6. GABA responses at stage 26 desensitize in a manner similar to that seen for mature cells, either with prolonged bath application of GABA or with repetitive ionophoretic application.

7. Nearly half of the cells tested at stage 26 respond to glycine, in concentrations as low as 5 μM. This sensitivity is absent by $3\frac{1}{2}$ d of development.

8. The responses of Rohon–Beard neurones to GABA are similar to those of other cells in that they involve a conductance increase, are mimicked by muscimol, and are blocked by picrotoxin. These responses are different in that they do not involve Cl^- and are blocked by low concentrations of curare.

9. Many of the characteristics of GABA receptors, i.e. the reversal potential,

desensitization, and pharmacology, are constant during development. However, the sensitivity of the cells to GABA and the spectrum of transmitters to which they are sensitive appear to change.

INTRODUCTION

It has recently been possible to determine the programmes of early functional cytodifferentiation for several populations of embryonic neurones developing *in vivo*. The development of electrical excitability and electrical uncoupling has been described for Rohon–Beard neurones, presumed primary sensory neurones of the frog spinal cord, and for the dorsal unpaired median (DUM) interneurones of the grasshopper thoracic ganglia (Baccaglini & Spitzer, 1977; Spitzer, 1982; Goodman & Spitzer, 1981). The development of neurotransmitter synthesis and electrical activity has been described for primary sensory antennal neurones of the moth (Hildebrand, 1980). Determination of the timing of these developmental changes makes possible further experiments designed to reveal any causal relationships among the various differentiated phenotypes.

Developmental studies of sensitivity to neurotransmitters have also been undertaken. The onset and development of chemosensitivity have been described for the grasshopper DUM neurones (Goodman & Spitzer, 1979, 1980). Among chordates, descriptions of the *in vivo* acquisition of transmitter sensitivity have been limited to the acetylcholine (ACh) sensitivity of skeletal muscle (Kullberg, 1974; Blackshaw & Warner, 1976*a*; Ohmori & Sasaki, 1977). These studies lend support to the notion that, while other differentiated phenotypes (e.g. electrical excitability) may change qualitatively during development, the chemosensitivity of excitable cells *in vivo* remains qualitatively the same once acquired. Thus, for the DUM neurones as well as in skeletal muscle, the ionic basis of the response seems to remain constant during development, although, at least in the case of muscle, there are significant quantitative changes in the sensitivity of the cells to transmitter.

In this paper we report on the acquisition of chemosensitivity by developing Rohon–Beard neurones of the frog spinal cord. We have found that the cells become sensitive to γ-aminobutyric acid (GABA) about embryonic stage 25, near the time when they become electrically uncoupled from each other and acquire a Na^+ current in their action potential. There is no tight correlation between the loss of coupling and the onset of chemosensitivity, but there may be between the acquisition of the GABA sensitivity and the Na^+-dependent component of the impulse. The GABA response differs significantly from those previously reported, in that it involves a conductance increase to Na^+ and K^+ but not to Cl^-, and is blocked by low concentrations of curare. The ionic basis, pharmacology, and desensitization characteristics of the GABA response do not change substantially during development. However, the sensitivity of the neurones to GABA does change with development. In addition, a significant fraction of the neurones studied at stage 26 responds to glycine, a sensitivity which the mature cells do not exhibit.

Preliminary accounts of some of these findings have appeared (Spitzer, 1976*a*; Bixby & Spitzer, 1981).

METHODS

Animals

Xenopus laevis tadpoles were obtained by conventional breeding procedures, and staged according to the criteria of Nieuwkoop & Faber (1956). Preparations were dissected and mounted in chambers as previously described (Baccaglini & Spitzer, 1977). The dissection we employed on stage 23–26 embryos was similar to that described by Spitzer (1982) for $\leqslant$ stage 25 embryos, except it was often helpful to separate overlying myotomal muscle from the dorsal spinal cord with an electrolytically sharpened tungsten needle. Rohon–Beard neurones were visualized with Zeiss–Nomarski interference contrast optics at 500 × magnification.

Recording conditions

Cells were impaled with glass capillary micro-electrodes (Hilgenberg Electrodenglas mit Innenfilament) filled with 3 M-potassium acetate (80–150 MΩ) or potassium chloride (40–90 MΩ). Tip potentials were less than 3 mV. In experiments involving changes in the ionic composition of the bathing solution, an agar bridge was used between the bath and the reference electrode. Currents and voltages were measured with a cathode follower amplifier having current injection capability (WP Instruments), and displayed on an oscilloscope or pen recorder (Gould Brush 220). For measurements of reversal potential we changed the cell's membrane potential in either direction by injection of small currents ($\leqslant 3 \times 10^{-10}$ A) through the micro-electrode. Conductances were measured with square pulses of hyperpolarizing current of 10^{-10} A or less. Electrodes were brought into balance prior to impalements, and data were rejected if the electrodes were no longer in balance for the same currents upon withdrawal from cells (see Spitzer, 1982). We found impalements of the embryonic cells to be more difficult than in older animals, and the resting potentials (r.p.s) of the cells varied considerably. We rejected data from cells having r.p.s less negative than −40 mV, and most cells in this study had r.p.s between −50 and −90 mV. Cells with low r.p.s were often held at −60– to −70 mV with steady hyperpolarizing current. Determination of the ionic dependence of a neurone's action potential was based solely on spike morphology, with long duration 'plateau' responses taken as Ca^{2+}-dependent and shorter 'peak and plateau' responses taken as Na^{+}- and Ca^{2+}-dependent (Baccaglini & Spitzer, 1977).

Solutions

The preparations were usually perfused continuously with amphibian saline, pH 7·4. The compositions of the normal saline and other saline solutions are listed in Table 1. For animals younger than stage 30, 10 mM-Mg^{2+} was added to the normal saline to reduce muscle contraction. In cases where a large amount of an ion was added or omitted (0 Na^{+}, high Ca^{2+}, low Cl^{-}), approximate tonicity was maintained by appropriately adjusting the molarity of the substituted or remaining ionic species. We added small amounts of blocking agents or other drugs without compensation for changes in osmolarity. The pH of the perfused drug solutions was kept above 7·0, as acidic pHs sometimes caused spurious changes in membrane voltage. With solutions containing catecholamines (10^{-4} M), we made up fresh solutions daily and bubbled the solutions with N_2 before the experiment, to prevent oxidation over the course of the day. All experiments were done at room temperature (~ 22 °C).

Drug application

Drugs were applied to the cells in three different ways. Often, they were simply perfused in known concentrations in the saline. A second method was the ejection of drug solutions from a blunt-tipped micropipette by pressure application to the butt of the electrode. Tip sizes of such 'puffer' electrodes ranged from 2 to 8 μm, achieved by abrasion on a frosted glass slide under visual control, and a system of valves, regulator, and pressure gauge (Clippard Insts.) was used to deliver pressures of 1–20 Lb./sq.in. With this microperfusion set-up, we assume that the concentration of drug reaching the cell surface is approximately that contained in the pipette, since bath and puffer application of the same GABA concentration gave similar results. Leakage of solutions did not appear to be a problem, since an electrode containing GABA could be brought down to the surface of an impaled cell without eliciting a response. Peptides delivered in this way were initially dissolved in 0·1 M-acetic acid and frozen; these solutions were then diluted at least 100-fold in normal saline containing 0·1 % bovine serum albumin to reduce binding of the peptides to the glass. Finally, in the case of GABA,

glycine, and ACh, we applied the drugs ionophoretically. Ionophoretic electrodes were the same size and shape as the recording electrodes, and not broken or bevelled. These electrodes had resistances of 1000–2500 MΩ when filled with 4 M-GABA, 2 M-glycine or 3 M-ACh. Because of this high resistance no braking current was considered necessary to prevent leakage. The ionophoretic pipette was often lowered while current pulses were being passed until it impaled the cell and a 'current response' was obtained (Harris, Kuffler & Dennis, 1971). We measured ionophoretic currents with a virtual ground (WP Instruments). D-Tubocurarine, picrotoxin, peptides, and other neurotransmitters were obtained from Sigma.

TABLE 1. Composition (mM) of salines used in perfusion

	NaCl	Na_2SO_4	Na isethionate	$CaCl_2$	KCl	HEPES	Tris HCl	TEA Cl
(1) Normal	125	0	0	10	3	5	0	0
(2) 0 Na^+	0	0	0	10	3	0	130	0
0 Na^+	0	0	0	10	3	5	0	125
low Cl^-	0	62·5	0	10	3	5	0	0
low Cl^-	0	0	125	10	3	5	0	0
high Ca^{2+}	95	0	0	40	3	5	0	0
high K^+	118	0	0	10	10	5	0	0

(3) Blocking agents (added to normal): TTX (10^{-6} g/ml) (Calbiochem); $CoCl_2$ (10 mM); $LaCl_3$ (0·5 mM), TEA Cl (20 mM).

RESULTS

Sensitivity to neurotransmitters

Bath application of GABA to mature Rohon–Beard cells (stages 46–49, 5–9 d old) produces a depolarization and an increase in membrane conductance (Fig. 1). A concentration of 100 μM-GABA produced a near-maximal response, 5 μM-GABA reliably elicited a response, and 1 μM gave no response. With applications of 100 μM-GABA, cells were depolarized by 15–40 mV from their resting potentials (r.p.s) of −70 to −90 mV. The response to bath-applied 100 μM-GABA reached a peak in 2–4 s, and declined to a plateau over a period of 25–30 s.

To determine whether the chemosensitivity of mature Rohon–Beard neurones is specific to GABA, we tested a variety of other neurotransmitters and neurotransmitter candidates with the same bath-application procedure. There was no detectable change in membrane potential or membrane conductance when the following drugs were applied to the neurones for 2–10 s at a concentration of 100 μM: norepinephrine, dopamine, 5-hydroxytryptamine (5-HT), histamine, octopamine, and glutamate ($n \geqslant 5$ for each agent). Of the thirty-four cells tested for responses to other transmitters, twenty-three were tested for response to 100 μM-GABA, and all of those responded with depolarizations and conductance increases. ACh could not be bath applied, since contractions of myotomal muscles would terminate the impalement. Therefore, we tested cells for ACh sensitivity by ionophoresis from micropipettes filled with 3 M-ACh. There was no effect of ACh on the Rohon–Beard cells, even with ionophoretic currents more than 10 × as large as those which gave easily detectable responses when applied to nearby muscle cells. Norepinephrine, GABA, and 5-HT have been found to affect the duration of Ca^{2+} action potentials in sympathetic and sensory neurones (Dunlap & Fischbach, 1978; Horn & McAfee, 1980). We tested these drugs (at a concentration

of 100 μM) for possible effects on the action potentials of Rohon–Beard cells, and found no effect on action potential duration or overshoot ($n = 3$).

Recent preliminary evidence suggests the presence of substance P and enkephalin in the dorsal spinal cord of larval *Xenopus*, and oxytocin in the area just beneath the skin (J. E. Lamborghini & H. J. Karten, personal communication). We therefore

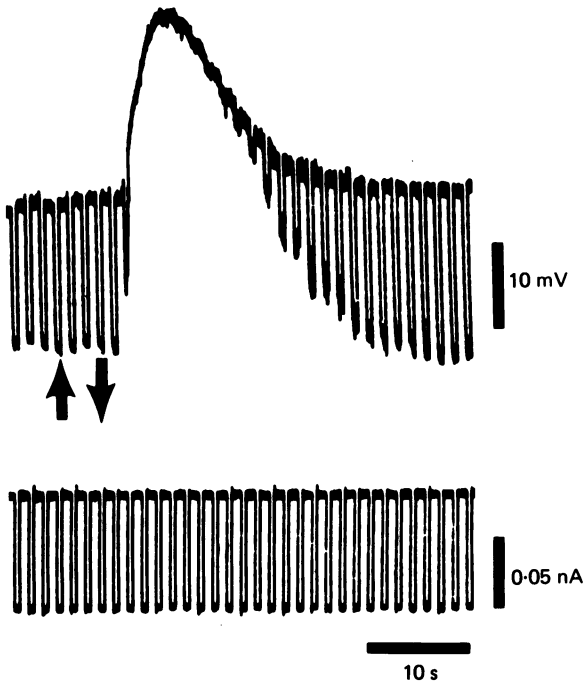

Fig. 1. Response of a mature Rohon–Beard neurone to bath application of 50 μM-GABA. GABA was applied for the time period bracketed by the arrows. A large conductance increase is associated with the response, as can be seen by the reduction in voltage change (upper trace) associated with the hyperpolarizing current pulses of constant amplitude (lower trace). The apparently long latency of the response is due to the lag time of the perfusion system. R.p. −80 mV; stage 48.

tested mature Rohon–Beard neurones for sensitivity to various putative peptide neurotransmitters. Because only small quantities of these substances were available, we applied them to the cells by the 'puffer' microperfusion technique. In control studies, we found that pressures of 1–20 Lb./sq. in. applied for 1–5 s to pipettes filled with 100 μM-GABA always produced responses in Rohon–Beard neurones. In contrast, none of the peptides tested produced detectable changes in membrane potential or conductance in these cells, during the minute following the puff. These peptides included met-enkephalin, substance P, oxytocin, bombesin, lys-vasopressin, arg-vasopressin, and vasotocin (concentrations $\geqslant 10$ μM; $n \geqslant 4$). In summary, we found no evidence for a sensitivity of mature Rohon–Beard cells to any neurotransmitter other than GABA. Thus the neurones' sensitivity to GABA is rather specific.

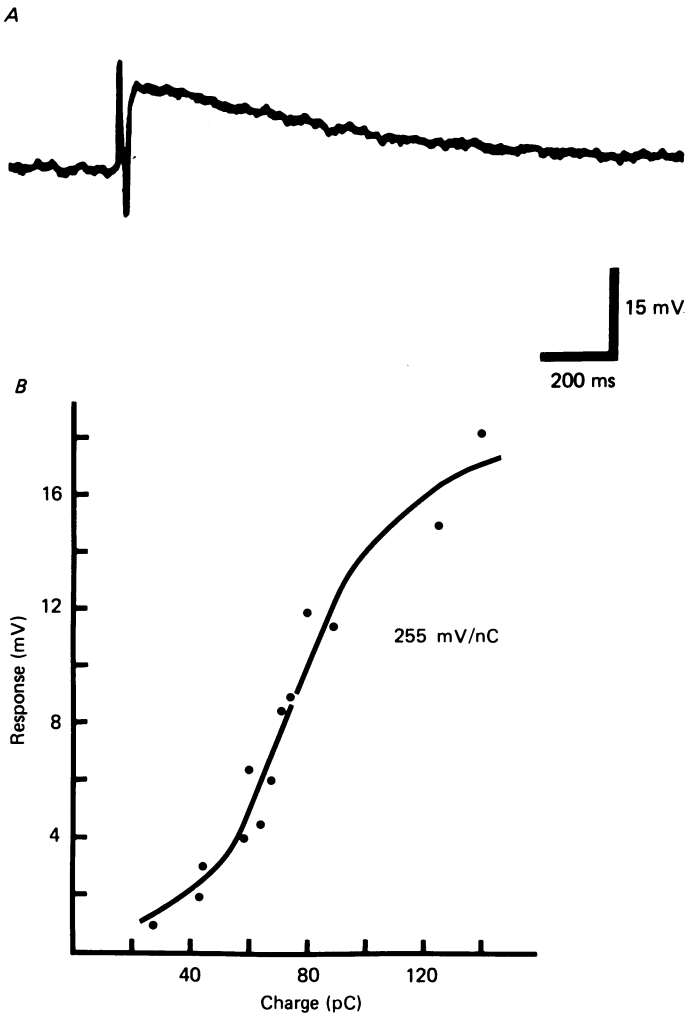

Fig. 2. *A*, response of a Rohon–Beard cell to ionophoretic application of GABA. The response rises in 15–20 ms, and decays in 800–900 ms. The ionophoretic pulse was 20 ms and 1·5 nA. R.p. −60 mV; stage 46/47. *B*, Dose–response curve for the ionophoretic application of GABA onto a mature Rohon–Beard neurone. The slope of the curve in the central (linear) region is 255 mV/nC. The curve was fitted by eye, but the slope in the linear region was determined by least-squares regression analysis. R.p. −86 mV; stage 46.

Onset of GABA chemosensitivity

Rohon–Beard cells tested at stage 22 showed no response to bath-applied 100 μM-GABA (n = 7). GABA chemosensitivity developed gradually over the next few hours of development, and was not uniform in any given preparation at this time. At stages 23–24, 23% of the cells tested responded with voltage and conductance changes to GABA (n = 26), and 46% responded at stage 25 (n = 13). By stage 26, nearly all

(86%) of the cells tested gave voltage and conductance responses to 100 μM-GABA ($n = 76$).

GABA dose–response relations

It is useful in many experiments to be able to apply transmitter focally to the cell surface, for example, to exclude the possibility that other methods of application are eliciting synaptically mediated responses. We found that with ionophoretic pulses of 20–30 ms in duration, the time-to-peak of the GABA-induced membrane depolarization varied from less than 20 to more than 300 ms in different cells (e.g. Fig. 2*A*). This variability may be due to inconsistency in our ability to appose the pipette directly to the cell surface, rather than to any differences in receptor kinetics.

Plots of membrane response (in mV) *vs.* ionophoretic charge ejected (in pC) gave graphs whose slopes in the linear region provided an estimate of GABA sensitivity (Fig. 2*B*). Sensitivities obtained in this way for mature cells ranged from 50 to 330 mV/nC in cells with a mean input resistance of 100 MΩ ($n = 11$). It is likely that the peak sensitivities of Rohon–Beard neurones are even higher than this, since we obtained single points as high as 700 mV/nC from some cells for which we did not obtain slope sensitivities. In some cases, we were able to sample several points on the soma of a single cell, and occasionally even 10–15 μm out on a process, but we saw no clear regions of higher or lower sensitivity. The initial curvature of dose–response curves suggests the presence of co-operativity in agonist binding to receptors (e.g. Fig. 2*B*); however, uncertainties in pipette characteristics and receptor geometry do not permit quantitative analysis.

Development of GABA sensitivity

The results discussed above show that Rohon–Beard neurones develop GABA chemosensitivity around stage 25, but that the exact timing of the acquisition of this property varies from cell to cell. Since stage 26 is the first stage at which most of the cells have acquired chemosensitivity, and since this is only 2 h after a significant fraction has become sensitive, we chose this stage to study the properties of the response when it first appears. The ionophoretic response at stage 26 is similar to that seen in older cells, with a similar range of rise times. The measured slope sensitivities of Rohon–Beard neurones at stage 26 ranged from 5 to 450 mV/nC ($n = 11$). These sensitivities are lower than, but largely within the range of, those of mature neurones. However, these measurements do not take into account the input resistances (R_{in}) of the cells, which change substantially during development (Baccaglini & Spitzer, 1977), or variations which might exist in the value of the recorded r.p. In accord with Baccaglini & Spitzer, we found the mean R_{in} of cells at stages 46–49 to be about 100 MΩ (mean ± S.E. of mean: 105 ± 13 MΩ; $n = 24$), while that of cells at stage 26 is four times as large (435 ± 63 MΩ; $n = 27$). Since these values differ significantly and since, especially at early times, individual values differ widely from each other, we normalized our sensitivity measurements. The sensitivity (mV/nC) was divided by the R_{in} (MΩ) to yield the sensitivity index (s.i.).

Providing that the shunt resistance of the channels opened by ionophoretic application of agonist is large compared to R_{in}, the conductance change caused by the agonist is proportional to the voltage measured (see Katz & Thesleff, 1957). Measurements of input resistance before and during

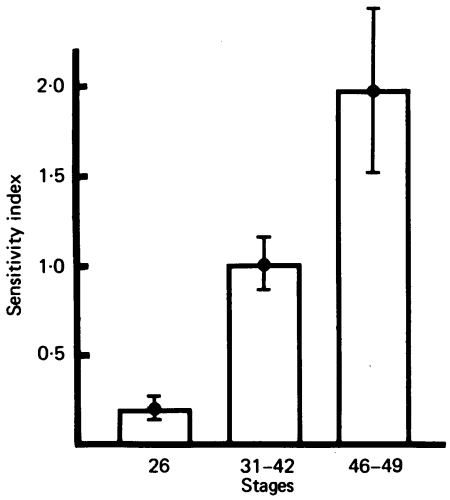

Fig. 3. Development of GABA sensitivity on the cell body of Rohon–Beard neurones. Histogram of the sensitivity index (sensitivity (mV/nC)/input resistance (MΩ)) of Rohon–Beard cells at various developmental ages. Each point is the mean ± S.E. of mean. corrected for differences in mean resting potential (see text). The index increases monotonically with age ($n = 6$, stage 26; $n = 13$, stages 31–42; $n = 10$, stages 46–49).

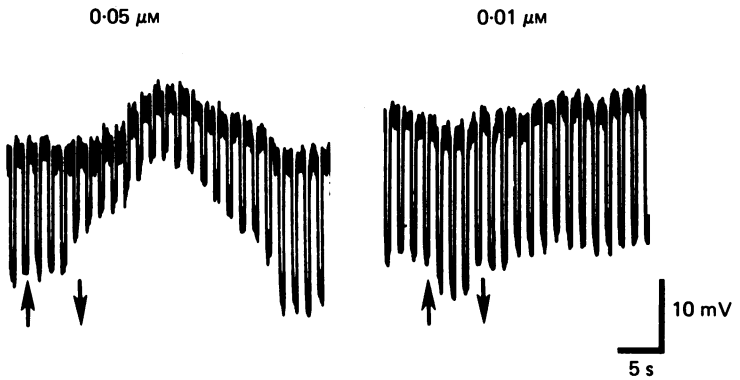

Fig. 4. Limits of GABA sensitivity of Rohon–Beard cells at stage 26. The same Rohon–Beard cell responds to bath application of 0·05 μM-GABA (left), but not to 0·01 μM (right). GABA was applied for the times indicated by the arrows. Current pulses: 0·02 nA; R.p. −60 mV.

ionophoretic responses indicate that this is the case for our experiments. Sensitivities can therefore be directly compared when each is divided by its respective R_{in}. In addition, since the driving force on the response is directly proportional to the reversal potential (V_r) minus the r.p., we corrected the mean values of the sensitivity index for each developmental age by multiplying by

$$\frac{V_r - V_m^*}{V_r - \overline{\text{r.p.}}},$$

where V_m^* is the standard membrane potential, which we arbitrarily took to be −80 mV, and $\overline{\text{r.p.}}$ is the mean r.p. for the cells whose sensitivity was determined at any given developmental age. This correction factor is small ($\leqslant 6\%$) for the cells in our experiments.

The s.i.s of cells measured at stage 26 ranged from 0·9 to 0·38, whereas the s.i.s of cells measured at stages 46–49 ranged from 0·15 to 5·2. We also determined ionophoretic sensitivities for cells at three intermediate stages: stages 31, 38, and 42. Since the s.i.s did not differ significantly among cells from these three stages, they were considered together, and the results were plotted in a developmental histogram (Fig. 3). We graphed the mean $\pm$ S.E. of the sensitivity index (corrected for r.p.) at each of the three periods. There is a progressive development of sensitivity, although it is not clear whether the maturation of this property is a continuous progression, or whether there are discrete changes at certain development ages.

When stage 26 Rohon–Beard cells were tested with different concentrations of bath-applied GABA, they seemed to fall into one of two classes. About half of the cells ($n = 7$) exhibited a sensitivity like that of the mature neurones, responding to 5 μM- but not to 1 μM-GABA. However, the other cells responded with voltage and conductance changes to concentrations as low as 0·1 μM or, more often, 0·05 μM-GABA (Fig. 4). These highly sensitive cells did not respond to 0·01 μM-GABA. There was a slight correlation between R_{in} and sensitivity, with the high sensitivity cells tending to have R_{in} values larger than those of the low sensitivity cells (517 ± 108 MΩ *vs.* 356 ± 71 MΩ). However, there were clearly some high sensitivity cells with R_{in} values in the low range (3/6).

Ionic basis of the GABA response

We determined the reversal potential (V_r) of the ionophoretic response by passing square current pulses of long duration through the recording electrode to change the cell's membrane potential (V_m), and applying GABA ionophoretically during the current pulse. In most V_r experiments, TTX, Co^{2+} and TEA^+ were added to the saline to reduce non-linearities in the current–voltage relation due to voltage-dependent channels. Even so, this relation was linear only over a restricted range for each cell, and we excluded data taken for values of V_m outside this linear region. As expected, we found that the GABA response became smaller when the cell was depolarized, and on several occasions the response could be seen to reverse in sign (Fig. 5*A*). However, for most cells the linear region of the *I–V* curve did not extend to the reversal potential, and we found this value by extrapolation (Fig. 5*B*). In mature cells, including two examined by small polarizations in saline without channel blockers, the extrapolated V_r was $-30·2 \pm 1·6$ mV ($n = 11$). This suggests the involvement of more than one ion in the response, since it would be unusual for a single ion to have such an equilibrium potential.

In the majority of GABA-sensitive cells studied, the GABA response has been attributed to a Cl^- current (Gallagher, Higashi & Nishi, 1978; Andersen, Dingledine, Gjerstad, Langmoen, & Mosfeldt Laursen, 1980). We examined the role of Cl^- in the GABA response of mature Rohon–Beard neurones by bathing cells in a solution containing only 28 mM-Cl^- and determining the reversal potential. Since many neurones have a significant resting permeability to Cl^-, which could eventually lead to a redistribution of the ion, we measured these reversal potentials by impaling cells within 30 s after starting perfusion with low Cl^- saline, and obtaining data for the V_r curves within 1–2 min after impalement. It seems unlikely that significant changes in internal Cl^- took place during this time (Barker & Ransom, 1978; Gallagher *et al.*

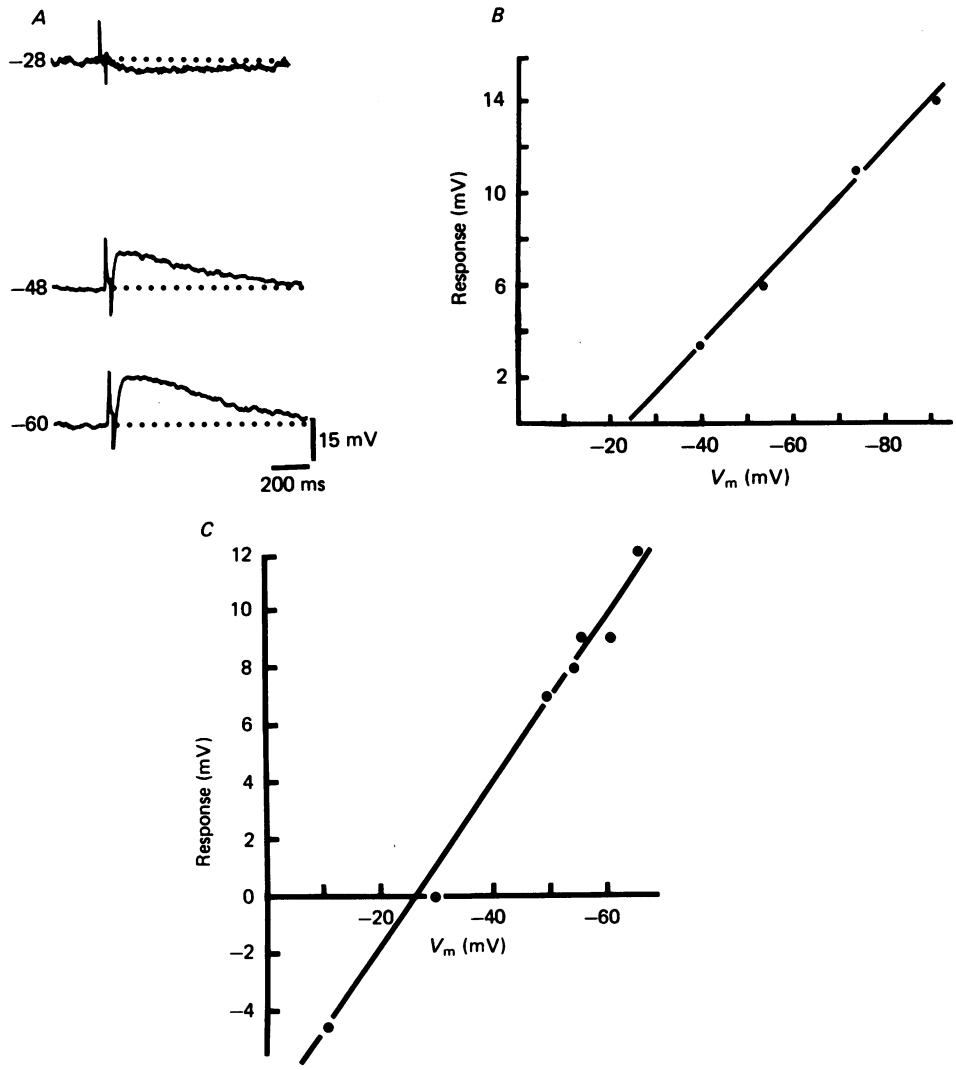

Fig. 5. The reversal potential of the GABA response in Rohon–Beard cells in normal saline. *A*, The ionophoretic response to GABA at three different membrane potentials in one mature cell. The response decreases with depolarization, and reverses in sign between −48 and −28 mV. Dotted lines indicate resting potential. Stage 46. *B*, graph of the ionophoretic GABA response *vs.* membrane potential for another cell. Only responses in the linear region of the cell's *I–V* relation are included. The extrapolated reversal potential (V_r) is −23 mV. Line is a least-squares fit to the data. R.p. −88 mV; stage 46. *C*, graph of the ionophoretic response *vs.* membrane potential for a stage 26 cell. The V_r is −26 mV. The line is a least-squares fit. R.p. −65 mV.

1978). In three cells examined in this way, the extrapolated V_r was $-28 \pm 3{\cdot}2$ mV (Fig. 6*A*), which is not different from the value for neurones in normal saline. This indicates that Cl^- is not involved in the GABA response in Rohon–Beard neurons.

In initial experiments designed to explore the contribution of Cl^- to the GABA response, we used the 'puffer' microperfusion technique to apply the low Cl^- saline solution directly onto an impaled cell. We found that application of low Cl^- saline to cells during repeated ionophoretic application

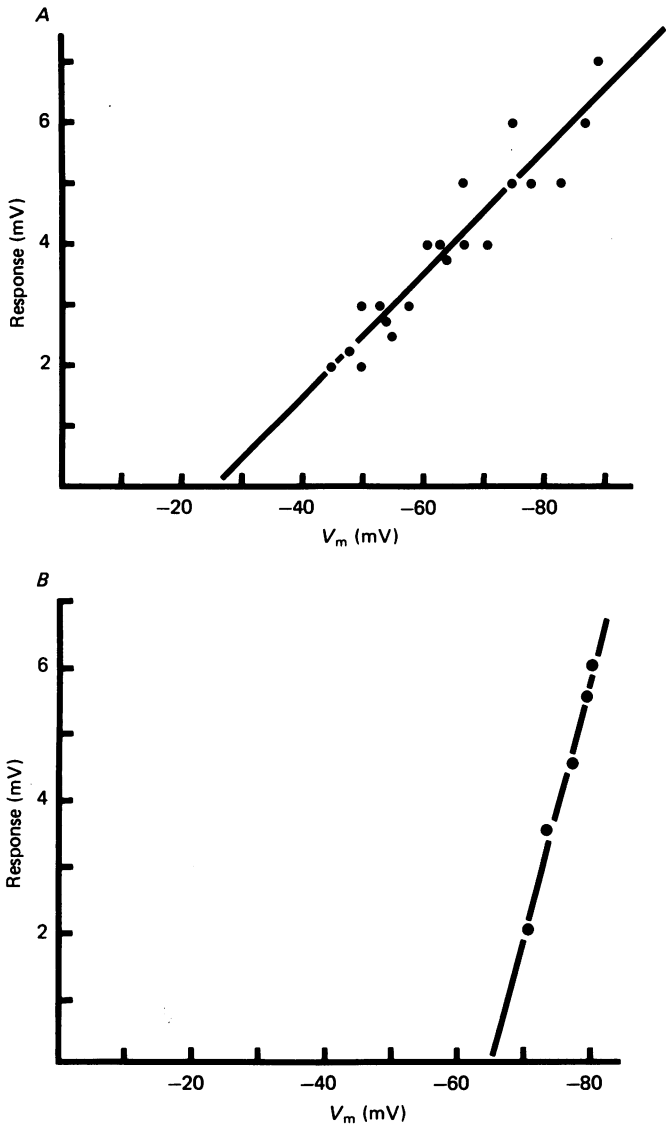

Fig. 6. Ionic dependence of the GABA response. *A*, ionophoretic response *vs.* membrane potential for a mature Rohon–Beard cell bathed in low Cl^- saline. Data points were taken within $2\frac{1}{2}$ min after the start of low Cl^- perfusion. The extrapolated V_r is -26 mV. The line is a least-squares fit to the data. R.p. -80 mV. *B*, ionophoretic response *vs.* membrane potential for a mature Rohon–Beard cell bathed in saline without Na^+. The extrapolated V_r is -65 mV. The line is a least squares fit. R.p. -80 mV.

of GABA resulted in a variable decrease in the size of the GABA response ($n = 3$). This would be an anomalous result if the normal depolarization caused by GABA were due to an efflux of Cl^- ions, since the driving force for Cl^- efflux would be increased with lower external Cl^- concentration. These effects were seen either with SO_4^{2-} or with isethionate substituted for Cl^-. The decrease in response magnitude could be explained by an increase in membrane conductance caused by the low Cl^- puff itself. To test this idea, we applied the low Cl^- solution to Rohon–Beard cells while measuring the membrane resistance. As predicted, application of the low Cl^- solution consistently caused a conductance increase of variable amplitude, usually associated with a slight (1–5 mV) hyperpolarization ($n = 8$). Control experiments showed that normal saline puffed onto cells bathed in low Cl^- solution resulted in a small ($\sim 10\%$) decrease in membrane conductance ($n = 3$), and that there was no effect of puffing normal saline onto cells bathed in normal saline ($n = 2$). A similar effect of anion substitution has been seen in locust muscle (Brookes & Werman, 1980) and cat primary afferent neurones (Gallagher *et al.* 1978). Whatever the mechanism, the effect of low Cl^- microperfusion on GABA responses can be explained by this direct effect on membrane conductance.

To test for the involvement of Na^+ ions in the GABA response, we measured the reversal potential in mature neurones bathed in saline having no Na^+ (substituting $Tris^+$ (four cells) or TEA^+ (one cell)) (Fig. 6*B*). The extrapolated V_r in these cells was $-63 \pm 2{\cdot}8$ mV (r.p. $= -73 \pm 5{\cdot}8$ mV), which is shifted in the hyperpolarizing direction from the value in normal saline. This suggests that Na^+ ions are carrying a significant fraction of the GABA current, and further, that another ion whose reversal potential is near the resting potential is also involved.

Since K^+ is the only other significant ionic species having an equilibrium potential near the resting potential, we suspected that it was carrying current through the GABA channels. Given the V_r in normal saline, and assuming that the GABA channels are permeable to Na^+ and K^+ and that the two ions permeate independently, one can calculate the relative conductances of the open channel to the two species (Takeuchi & Takeuchi, 1960):

K^+ equilibrium potential (E_K) = r.p. $= -88$ mV (Spitzer, 1976*b*);

assume $E_{Na} = +55$ mV.

Then $$V_r = \frac{g_{Na}E_{Na} + g_K E_K}{g_{Na} + g_K} = -30 \text{ mV and } g_{Na}/g_K = 0{\cdot}7.$$

Accordingly the V_r expected in saline containing 10 mM-K^+ ($E_K = -58$ mV) is $-11{\cdot}5$ mV.

To test this prediction, we determined reversal potentials for mature neurones in 10 mM-K^+ saline. In five cells examined in 10 mM-K^+, the V_r was $-15 \pm 3{\cdot}7$ mV, which is in reasonable agreement with the predicted value. The range of membrane voltages from which points could be taken was small for these experiments (due to non-linearities in I–V plots), and thus this extrapolation was subject to more error than the others. However, the agreement is close enough to suggest strongly that the GABA responses in Rohon–Beard cells are due to channels permeable to Na^+ and K^+, in a g_{Na}/g_K ratio of approximately 0·7.

The size of the GABA response at constant V_m was unaffected by bathing the cells in a solution containing 4 × normal Ca^{2+}, which suggests that Ca^{2+} influx does not contribute to the response.

To determine whether the ionic basis of the Rohon–Beard cell's GABA response changes during development, we measured the V_r in neurones at stage 26, shortly

after the response can first be elicited. The V_r at stage 26 was $-28{\cdot}5 \pm 2{\cdot}9$ mV ($n = 8$), which is not significantly different from that measured in mature cells (Fig. 5*C*). This equivalence suggests that the dependence of the response on Na^+ and K^+ is constant during development, since any change in one ionic conductance would have to be exactly counterbalanced by a change for some other ion(s) to give this result.

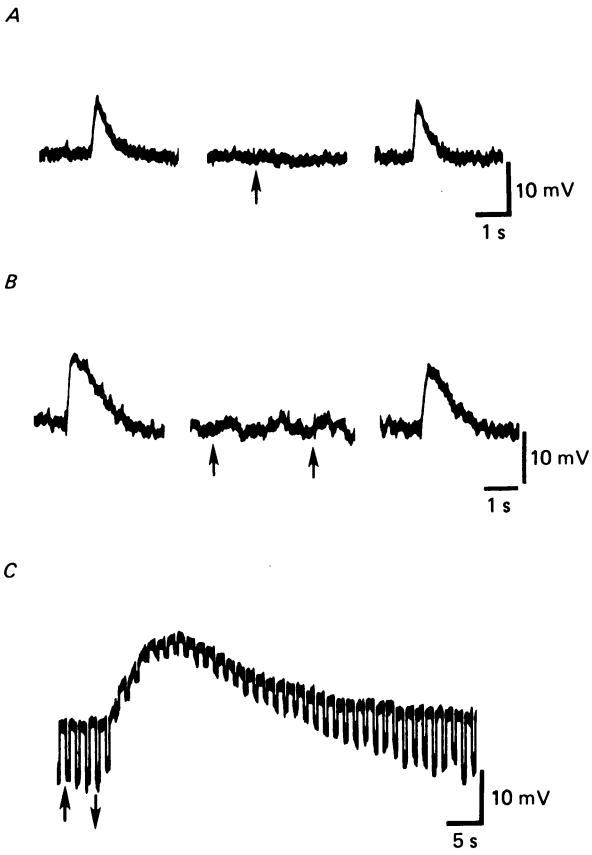

Fig. 7. Pharmacology of the GABA response in mature Rohon–Beard cells. *A*, bath application of 10 μM-picrotoxin for 1 min abolishes the ionophoretic response to GABA, which returns to normal after a wash of 1½ min. Arrow indicates time of ionophoretic pulse. R.p. −84 mV; stage 47. *B*, application of 50 μM-curare for 30 s abolishes the ionophoretic GABA response in another cell; the response returns after a wash of 25 s. Arrows indicate times of ionophoretic pulses. In other cells, the response could be completely abolished by 10 μM-curare, but only with longer durations of application. R.p. −82 mV; stage 47. *C*, bath application of 10 μM-muscimol (arrows) produces a 15 mV depolarization and a large conductance increase in a third cell. 0·07 nA current pulses; r.p. −85 mV; stage 48.

Pharmacology and desensitization of GABA responses

Ionophoretic responses to GABA in mature cells could be blocked reversibly by bath application of picrotoxin, a common inhibitor of GABAergic transmission (Fig. 7*A*). Complete blockade was always achieved with 50 μM ($n = 5$), and usually with 10 μM (three of four cells). The responses were unaffected by strychnine, a blocker

of glycine function, at 10 μM ($n = 4$), and were reduced slightly (33%) by 50 μM-strychnine ($n = 2$). GABA responses could also be antagonized by curare, which completely blocked responses at 50 μM ($n = 7$), and usually at 10 μM (two of three cells) (Fig. 7*B*). However, neither α-bungarotoxin nor bungarotoxin 3·1, a blocker of neuronal ACh receptors (Ravdin & Berg, 1979), blocked the GABA response following

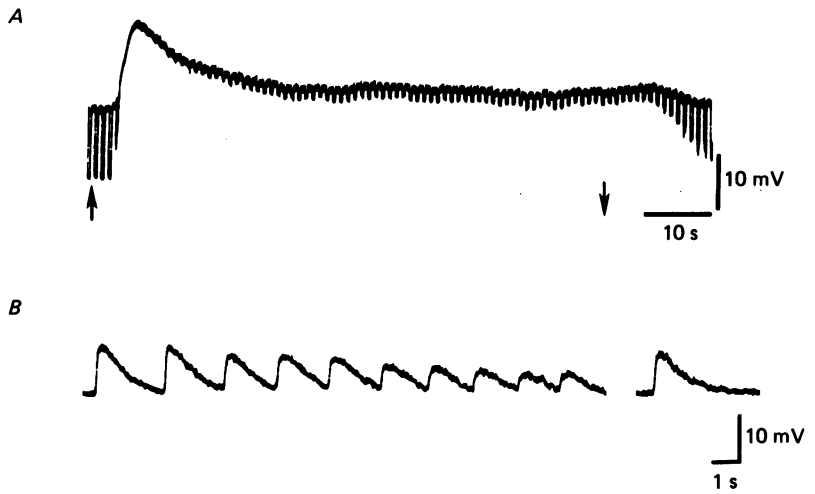

Fig. 8. Desensitization of the GABA response in mature Rohon–Beard neurones. *A*, the voltage and conductance change decline to a steady-state value during constant bath application of 50 μM-GABA. 0·08 nA current pulses; stage 48. *B*, repeated ionophoretic pulses of GABA result in a gradual decline of the response, until a steady-state response is approached. The response returns to nearly its initial size after 9 s. R.p. −90 mV; stage 49.

a 1 h incubation at a concentration of 2×10^{-8} M. In addition, blockers of voltage-dependent ionic channels (Co^{2+}, La^{3+}, TEA^{+}, TTX) had no effect on GABA responses. Muscimol, a GABAergic agonist, seems to be somewhat more effective than GABA on Rohon–Beard cell receptors, since responses could be elicited by bath concentrations as low as 1 μM. Muscimol produced depolarizations and conductance increases with time courses similar to those produced by GABA (Fig. 7*C*). The pharmacology of the Rohon–Beard cell GABA response is essentially the same at stage 26 as it is in mature cells, 5–8 d later in development. Evaluation of comparable numbers of neurones revealed the same sensitivities to the various pharmacological agents; bungarotoxins were not tested. Abolition of the response at stage 26 was more rapid than the effects of these drugs on mature cells, probably because of the absence of diffusion barriers in the younger spinal cords.

Mature Rohon–Beard cell receptors desensitize in response to GABA, as seen by the decline in response with constant bath application (Fig. 8*A*). It has been proposed that prolonged bath application can lead to depression of responses through changes in intracellular ion concentrations (Adams & Brown, 1975), or through activation of a transmitter uptake system (Krnjević, Puil, & Werman, 1977), rather than true desensitization. We therefore investigated this phenomenon by the ionophoretic application of agonist. Repeated application of 40 ms pulses of GABA to the cells

resulted in a depression of the response in most cases (ten out of thirteen), which approached a steady state after seven to twenty pulses, and returned to approximately the initial level after 7–10 s (Fig. 8*B*). The extent of desensitization varied from cell to cell, and a few cells could be demonstrated to desensitize only with much longer pulses (up to 200 ms; two out of thirteen), or with bath application (one out of

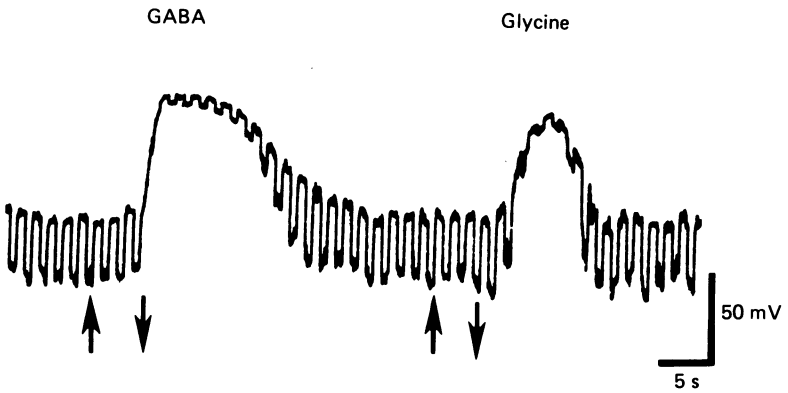

Fig. 9. GABA and glycine responses in a stage 26 Rohon–Beard neurone. The cell depolarizes to approximately the same level in response to 100 μM-glycine (right) as with 100 μM-GABA (left). The glycine response decays more rapidly, even though the two drugs were applied for approximately the same time (arrows). Current pulses 0·10 nA; r.p. −85 mV. Note the low voltage gain.

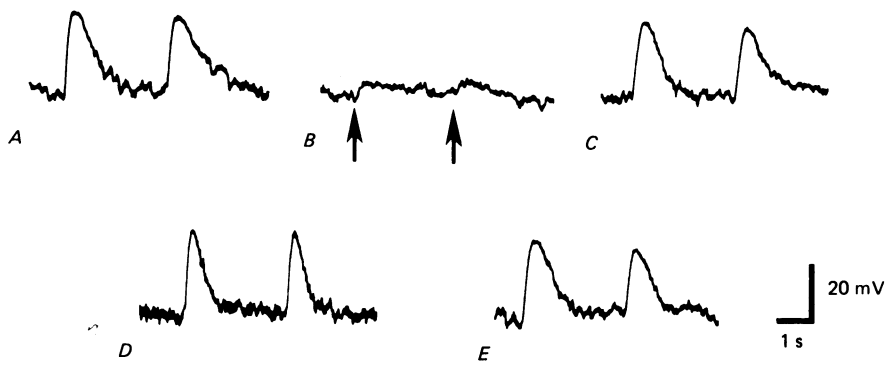

Fig. 10. Pharmacology of the glycine response of newly sensitive Rohon–Beard cells. *A*, normal ionophoretic response to glycine, stage 26. *B*, bath application of 1 μM-strychnine abolishes the glycine response after 13 s. *C*, the response returns after a 35 s wash. *D*, bath application of 50 μM-picrotoxin for 45 s has no effect on the glycine response. *E*, bath application of 50 μM-curare for 40 s has no effect. Arrows indicate time of glycine pulses. All records from one cell; r.p. −65 mV.

thirteen cells). As is the case for other receptors (Adams, 1975; Anis, Clark, Gration, & Usherwood, 1981) the rate of desensitization and its magnitude depended on the dose of drug, which could be seen either by increasing ionophoretic pulse duration or by bathing cells in different concentrations of GABA (data not shown). Rohon–Beard cells also desensitize in response to prolonged bath application of muscimol. Just as

for the mature cells, desensitization of stage 26 neurones was apparent with either bath or ionophoretic application of GABA; the time course of desensitization and its recovery were not obviously different from those in mature cells ($n = 6$). Desensitization is therefore apparent in essentially mature form as soon as receptors can be physiologically detected in the neuronal membrane.

Transient sensitivity to glycine

Rohon–Beard cells at stage 26 are also insensitive to the other neurotransmitter candidates that we tested on mature neurones (see above); each agent was tested on at least five neurones. However, a large fraction of the neurones examined at stage 26 gave vigorous responses to bath application of 100 μM-glycine (seventeen out of thirty-eight cells with GABA responses; Fig. 9), a substance which never elicited a response in mature Rohon–Beard neurones ($n = 14$, for stages 46–47). It seems likely that these cells become sensitive to glycine and GABA at the same stage of development, since no responses to glycine were seen at stages 23–24 ($n = 5$), and no cells at stage 25–26 were found which responded to 100 μM-glycine but not to 100 μM-GABA ($n = 9$). In cells responding to GABA and glycine, both agonists produced depolarizations and conductance increases which were of comparable magnitude. Cells tested responded to concentrations as low as 10 μM ($n = 1$) or 5 μM ($n = 3$) suggesting that the glycine and GABA sensitivities of these cells may be similar. However, glycine responses were consistently shorter than GABA responses for the same duration of application. The R_{in} and r.p. for glycine-sensitive cells were not obviously different from those for cells insensitive to glycine. We thought it possible that the cells responding to 100 μM-glycine at stage 26 were those which are sensitive to very low GABA concentrations, but two neurones which responded to 25 μM-GABA, but not to 0·25 μM, showed good responses to 100 μM-glycine and, conversely, three cells responded to 0·4 μM-GABA but not to 100 μM-glycine.

Stage 26 Rohon–Beard cells could also respond to glycine applied ionophoretically (Fig. 10). The ionophoretic glycine response, in contrast to the GABA response, was unaffected by 10 μM-picrotoxin ($n = 5$), 50 μM-picrotoxin (only one cell tested), or 50 μM-curare ($n = 2$). The responses could be reversibly abolished, however, by 10 μM-strychnine ($n = 3$), and, in the one case tested, by 1 μM-strychnine. The reversal potential and desensitization of the glycine response were not investigated. We do not know when this glycine sensitivity disappears from the Rohon–Beard cell population; however, it is absent by stage 43 ($3\frac{1}{2}$d old; $n = 14$).

Relationship of chemosensitivity to other developing membrane properties

Rohon–Beard neurones become uncoupled around stage 25, and we thought it possible that this event is causally related to the onset of chemosensitivity. Coupling among Rohon–Beard cells is voltage-dependent, and evidence has been presented that the presence of a spontaneous voltage transition together with a large increase in input resistance during a constant current pulse indicates that an impaled cell has become transiently uncoupled from its neighbours (see Spitzer, 1982). It is thus possible to assay for the presence of coupling with single micro-electrode impalements, and test for the concurrence of developmental uncoupling and onset of chemosensitivity by asking whether coupled cells are insensitive to GABA while uncoupled cells are sensitive.

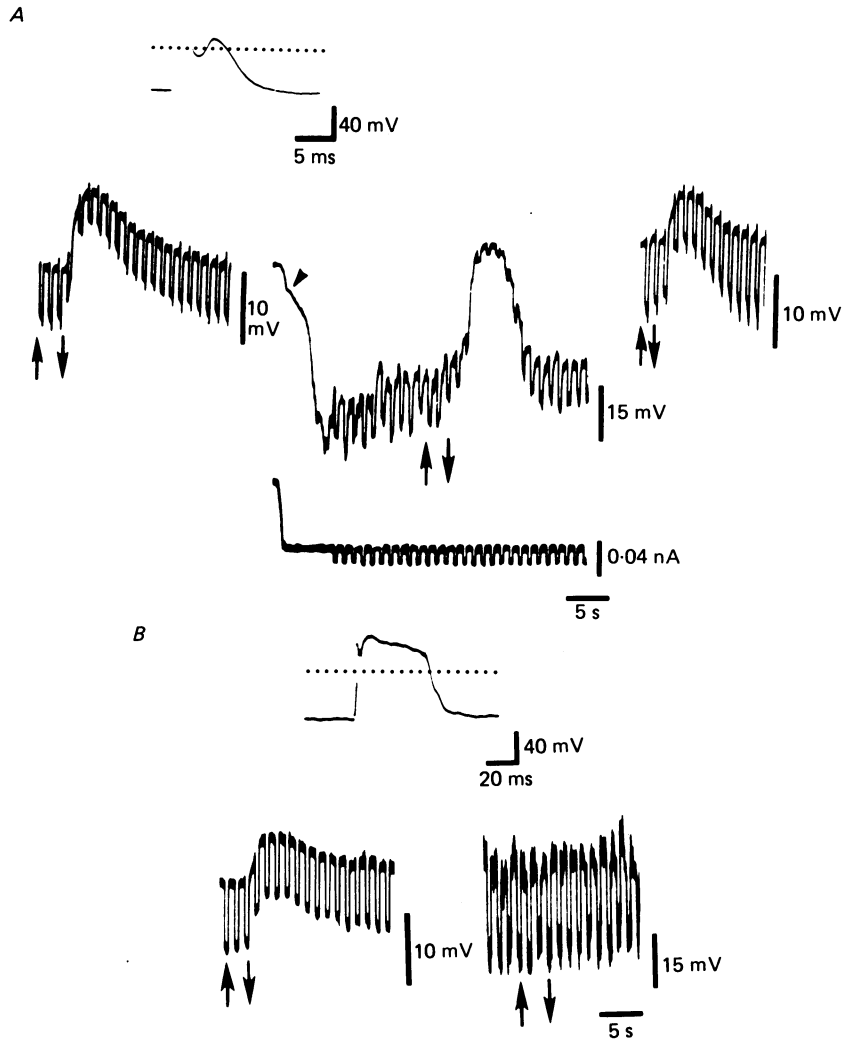

Fig. 11. GABA responses in electrically coupled Rohon–Beard cells. *A*, a neurone with a Na^+/Ca^{2+} action potential (upper left; dotted line 0 mV; depolarizing current pulse not shown, upstroke partially lost because of imbalance of electrode during depolarizing current pulse) responds to bath application of 100 μM-GABA with a depolarization and a conductance increase (lower left; current pulses not shown, 0·1 nA). When a long-duration 0·1 nA hyperpolarizing current step was passed through the recording electrode, the cell uncoupled from other cells, as indicated by the spontaneous transition in membrane voltage (arrowhead, lower middle). In this condition, pulses of 0·02 nA gave 9–10 mV voltage changes, indicating a large decrease in cell conductance due to uncoupling. Application of GABA to the uncoupled cell resulted in a conductance increase and a large depolarization, consistent with the increased driving force due to the steady hyperpolarization. When the cell was allowed to recouple by turning off the 0·1 nA current step, the GABA response returned to its initial form (lower right; 0·1 nA current pulses). Stage 24; r.p. −48 mV. *B*, a neurone with a Ca^{2+} action potential (upper trace; dotted line 0 mV; depolarizing current pulse not shown) responds to bath application of 100 μM-GABA with a depolarization, but no detectable conductance change (lower left; 0·07 nA current pulses). This cell was uncoupled from its neighbours with a hyperpolarizing current step, as in *A*. Application of GABA to the uncoupled cell produced no detectable response (lower right) even though the cell was hyperpolarized by 40 mV, and exhibited a large input resistance (0·05 nA current pulses). The impalement was lost before the cell could be recoupled and tested again, but in four other cases like this, the response returned to its initial form upon recoupling. Stage 25; r.p. −60 mV.

We found that the acquisition of chemosensitivity can precede the loss of coupling, since coupling can be present in a neurone which is demonstrably sensitive to GABA, and insensitive cells can be shown to be coupled to at least one other sensitive cell. Cells that showed voltage and conductance changes to bath-applied GABA at stages 23–25 were hyperpolarized to see if they could be transiently uncoupled from their neighbours. The spontaneous voltage transition during the hyperpolarizing pulse and the increase in input resistance indicated that uncoupling had occurred (Fig. 11*A*), as previously demonstrated. The voltage response of the cell to GABA in the uncoupled state was larger, consistent with the higher input resistance and more hyperpolarized membrane potential. The response returned to roughly its original form when the cell was allowed to recouple to its neighbours ($n = 3$). Other cells studied at the same developmental stages gave GABA responses but had lost the feature of voltage-dependent coupling ($n = 7$).

We often recorded voltage responses upon bath application of GABA, with little or no concomitant conductance change (Fig. 11*B*). These responses could be accounted for if the impaled cells themselves were insensitive to GABA, but were electrically coupled to sensitive cells. This possibility was supported by the consistent finding that cells of this class gave no response to GABA while uncoupled by steady hyperpolarizing current pulses, in spite of the increased input resistance of the cells and driving force on the ions whose conductances are activated by GABA (Fig. 11*B*; $n = 6$; the presence or absence of coupling was not tested in a further eight cells, in which the responses presumably arose in the same way). In addition, the converse result was obtained, in which neurones that had not yet acquired a GABA response had nevertheless lost their voltage-dependent coupling (five out of eleven cells with no response to 100 μM-GABA; stages 24–26). These results indicate that loss of voltage-dependent coupling and acquisition of chemosensitivity are independent events which are roughly coincident in time.

Rohon–Beard cells also acquire the Na^+ component of their action potential around stage 25 (Baccaglini & Spitzer, 1977). This can be seen by the characteristic shape of the action potential, which has an initial peak (Na^+ component) followed by a plateau (Ca^{2+} component). We studied the correlation of electrical coupling with the appearance of the Na^+ current using the coupling assay described above. Of the neurones examined at stages 25–26, four out of eight cells with Ca^{2+} spikes were still coupled to other cells, and two out of four cells with Na^+/Ca^{2+} spikes were still coupled. Thus there is no obligatory relationship between loss of coupling and the appearance of the inward Na^+ current (see also Spitzer, 1982).

When the relationship of the acquisition of chemosensitivity to that of inward Na^+ current was analysed, a different pattern emerged. In cells examined at stages 24–26, only two out of thirty-one cells which showed long-duration Ca^{2+} spikes exhibited GABA chemosensitivity, as measured by voltage and conductance responses to bath-applied 100 μM-GABA. In contrast, ten of the twelve cells with Na^+/Ca^{2+} spikes gave voltage and conductance responses to GABA. This correlation is suggestive of a relationship between the two events.

DISCUSSION

General properties of the GABA response

The GABA responses of Rohon–Beard neurones are likely to be physiologically relevant. The sensitivities of these cells, as measured either with bath application (minimal concentration for response, $\leqslant 5\ \mu$M) or by ionophoresis (sensitivity > 300 mV/nC) are as high or higher than GABA sensitivities reported for other neurones (Adams & Brown, 1975; Dichter, 1980; Choi & Fischbach, 1981), where GABA clearly has a functional role. In addition, of a host of other putative neurotransmitters tested, none elicited a detectable voltage or conductance change when bath applied to mature Rohon–Beard cells. Most other mature neurones examined have been found to respond to more than one neurotransmitter.

Ion substitution data indicate that a mixed Na^+/K^+ current is involved in the GABA response in Rohon–Beard neurones, with K^+ as the predominant charge carrier. This is strong evidence that GABA can act on vertebrate neurones to cause cationic conductance increases, which could be functionally excitatory. GABA responses in other vertebrate neurones, by contrast, have been found to involve increases in Cl^- conductance, whether they are depolarizing or hyperpolarizing (Nishi, Minota, & Karczmar, 1974; Adams & Brown, 1975; Gallagher *et al.* 1978; Barker & Ransom, 1978; Choi & Fischbach, 1981).

The GABA responses of Rohon–Beard neurones are blocked by low (10 μM) concentrations of picrotoxin, as is the case for responses in some other neurones (Gallagher *et al.* 1978; Dichter, 1980). Since these other responses are Cl^--dependent and the Rohon–Beard cell response is mediated by Na^+ and K^+, it is likely that picrotoxin in these systems is acting on the receptor, rather than by interfering directly with the associated ion channels. In other systems, picrotoxin is reported to block GABA responses at concentrations ranging from 100 μM to 1 mM (Barker & Nicoll, 1973; Choi & Fischbach, 1981), and in these cases may be acting directly on Cl^- channels (Takeuchi & Takeuchi, 1969; Yaroswky & Carpenter, 1978). A possible dual action of picrotoxin on GABA responses has some precedent, since, in skeletal muscle, curare has been found to act both on ACh receptors and, at somewhat higher concentrations, on the receptor-associated ionic channels (Colquhoun, Dreyer & Sheridan, 1979). The GABA responses in these vertebrate spinal cord neurones can be blocked completely by 10 μM-curare. To our knowledge, this is the first report of GABA-blockade by such low concentrations of curare for any neurones.

Receptor properties during development

The fact that the V_r of the GABA response is the same at stage 26 as in mature cells suggests strongly that the ionic basis of the GABA response is constant during development. This finding is in line with earlier results on ACh receptors in skeletal muscle (Blackshaw & Warner, 1976*a*; Ohmori & Sasaki, 1977) and ACh and GABA receptors in grasshopper neurones (Goodman & Spitzer, 1979, 1980). The lack of a developmental change in the ionic basis of the response to transmitter may be a rather general phenomenon, since it has now been found for vertebrate neurones as well as skeletal muscle and invertebrate neurones.

Rohon–Beard neurones at stage 26 exhibit the same sensitivity to GABA-blockade

by curare and picrotoxin as the mature cells, and depolarize in response to muscimol. Desensitization of these GABA receptors also is apparent at stage 26, and appears similar to that seen at late stages, though changes in the rate and/or magnitude of desensitization could have been missed in our experiments. There is little information available on the presence or absence of developmental changes in pharmacology or desensitization of other transmitter receptors, and what is known is mainly confined to skeletal muscle. Curare, a potent antagonist of ACh action in adult skeletal muscle, has been reported to depolarize embryonic rat muscle fibers (Ziskind & Dennis, 1978). However, neither these curare responses of embryonic muscle nor the responses of rat myotubes *in vitro* to bath-applied ACh appear to desensitize (Ritchie & Fambrough, 1975; Ziskind & Dennis, 1978), though the ACh responses of adult muscle cells certainly do (e.g. Thesleff, 1955).

Development of GABA sensitivity

There appears to be a progressive development of ionophoretic sensitivity on the somata of Rohon–Beard neurones, such that normalized sensitivities averaged 10-fold higher at stages 46–49 than at stage 26. This suggests that the average density of somal GABA receptors increases during this time, or that there is an increase in the single channel conductance or mean channel open time. We believe this is a true development, rather than a technical artifact, for the following reasons. Although the impalements are generally more difficult at early stages due to poor visibility and fragility of the neurones, they are no easier at stage 31 than stage 26, or at stage 46 than stage 42 and, furthermore, the resting potentials of the cells from which we obtained ionophoretic data averaged at least -75 mV for all the time periods studied. Secondly, we had instances at all ages in which the ionophoretic electrode impaled the cell, giving a direct response to injected current, which indicates that our pipette was often very close to the somal membrane. The similarity in the range of rise times at all stages also suggests that proximity to the cell did not vary systematically with age. Finally, the long times necessary for pharmacological blockade at late stages, coupled with our observations through the microscope, suggest that diffusion barriers which might lead to artifactually low sensitivities are more of a problem at late stages than at stage 26.

In contrast to the developmental increase in ionophoretic sensitivity, the sensitivity of the cells measured by bath application of GABA does not seem to increase substantially during the developmental period studied. Interpretation of these results is confounded by the presence of desensitization and diffusion barriers, as well as our lack of information on the geometry or membrane properties of neuronal processes, the presence or absence of electrical coupling in the cells tested, and the distribution of receptors at locations distal to the soma.

It is difficult to compare our results on ionophoretic sensitivity with those found for the development of other systems, since we believe the somal GABA receptors to be non-synaptic, and have no information on the possible accumulation or loss of receptors elsewhere in the membrane. Embryonic muscle fibres, though, generally accumulate ACh receptors at junctional sites and lose receptors elsewhere as they mature (Diamond & Miledi, 1962; Blackshaw & Warner, 1976*a*; Burden, 1977; Bevan & Steinbach, 1977; Ohmori & Sasaki, 1977).

Transient sensitivity to glycine

About 45% of the neurones we examined at stage 26 were depolarized by bath-applied glycine, a response which was not seen in mature cells. This depolarization was generally comparable in magnitude to that caused by the same concentrations of GABA on the same cells, suggesting that the reversal potentials of the two responses may be similar. The different pharmacology of the glycine and GABA responses suggests that the two amino acids are not acting on the same receptor, although this evidence is not conclusive (Diamond & Roper, 1973). If, as seems likely, responses to glycine and GABA appear simultaneously, this would be similar to the simultaneous appearance of responses to ACh and GABA in the DUM neurones (Goodman & Spitzer, 1979, 1980).

The presence or absence of glycine sensitivity at stage 26 defines two populations of Rohon–Beard cells. It is unlikely that the glycine-sensitive cells are displaced extramedullary cells, since these constitute a small minority of the early neurones of the *Xenopus* spinal cord (Lamborghini, 1980). The disappearance of glycine sensitivity could in principle be due either to a change in transmitter sensitivity in individual neurones or to the selective death of those neurones with glycine receptors. However, such a selective death seems unlikely in view of the fact that Rohon–Beard cell death has not begun by stage 43 (Lamborghini, 1981), a time at which we were unable to find neurones sensitive to glycine. The developmental significance of this transient transmitter sensitivity is unknown.

Developmental timetables

Rohon–Beard neurones consistently begin to respond to GABA around stage 25, although acquisition of chemosensitivity, like other differentiated properties of these cells, is not a synchronous event in all cells of an embryo. The neurones retain this sensitivity at least until the onset of cell death in the population (Lamborghini, 1981). Stage 25 is also the time when two other membrane properties change: the neurones begin to exhibit a Na^+-dependence to their action potentials and become electrically uncoupled from each other. It has been proposed for some time that gap junctions, the mediators of electrical coupling, may in some fashion help to regulate development, especially in the nervous system (see Griepp & Revel, 1977; Bennett & Goodenough, 1978), and the temporal correlation between loss of coupling and various forms of differentiation in several systems is certainly suggestive (Potter, Furshpan & Lennox, 1966; Blackshaw & Warner, 1976*b*; Dennis, Ziskind-Conhaim & Harris, 1981; Goodman & Spitzer, 1979, 1981). However, the evidence from our study, as in these other systems, is consistent with the idea that the loss of coupling is only coincidentally associated with the acquisition of these specific phenotypes. It remains possible that other differentiated properties are intimately associated with the loss of electrical coupling.

There is a correlation between acquisition of GABA chemosensitivity and the Na^+ current in the action potential of Rohon–Beard neurones. The two events may be even better correlated than our results suggest, since those few cases which did not fit the general pattern might be due to our failure to detect a small Na^+ component in a largely Ca^{2+}-dependent spike or to the artifactual loss of chemosensitivity in an

impaled cell. In any event, whether acquisition of the voltage-dependent Na^+ channels and the GABA receptors occurs with any kind of obligatory relationship in these neurones is in principle a testable proposition.

It has been possible in a few cases to study the initial acquisition of neuronal phenotypes *in vivo*, but so far, easily comparable developmental timetables exist only for Rohon–Beard cells and two populations of grasshopper neurones (Spitzer & Lamborghini, 1981; Goodman & Spitzer, 1979; Goodman, Bate, & Spitzer, 1981). The findings presented here provide an opportunity to assess the generality of such timetables, in particular with respect to the acquisition of chemosensitivity. As in Rohon–Beard neurones, developmental changes in chemosensitivity have been found to be associated with changes in electrical excitability in other cells (reviewed by Spitzer, 1979). However, the onset of chemosensitivity, which in Rohon–Beard neurones occurs after the onset of electrical excitability, is a very early phenotype in the grasshopper DUM neurones, and takes place some days earlier than the acquisition of the impulse. Other discrepancies in the timing of differentiation exist for those few cases in which a comparison is possible (see Spitzer & Lamborghini, 1981). Nevertheless, in two neuronal populations and in skeletal muscle, acquisition of chemosensitivity and electrical excitability can occur while cells are still electrically coupled. Thus there may be some general features in the development of excitable membranes, while the detailed developmental timetables vary from one cell population to another.

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