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Responses to GABA, glycine and β-alanine induced in *Xenopus* oocytes by messenger RNA from chick and rat brain

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Poly $(A)^+$ messenger RNA (mRNA) was extracted from rat and chick brains, and injected into oocytes of *Xenopus laevis*. This led to the expression of receptors that evoked membrane currents in response to γ-aminobutyric acid (GABA), glycine and β-alanine. These currents all inverted at about the chloride equilibrium potential in the oocyte, and showed a marked rectification at negative potentials.

Oocytes injected with mRNA from chick optic lobe gave large responses to GABA and β-alanine, but small responses to glycine. In contrast, one fraction of mRNA from rat cerebral cortex (obtained by sucrose density gradient centrifugation) caused oocytes to develop sensitivity to GABA, glycine and β-alanine, whereas a different fraction induced sensitivity to glycine and β-alanine, but very little to GABA. The pharmacological properties of the three amino acid responses also differed. Barbiturate and benzodiazepines potentiated the responses to GABA and β-alanine, but not to glycine. Strychnine reduced the responses to glycine and β-alanine, but not to GABA, whereas bicuculline reduced the responses to GABA and β-alanine, but not to glycine. We conclude that different species of mRNA code for receptors to GABA and glycine, and possibly also for separate β-alanine receptors.

Introduction

We have previously shown that the brains of various animal species contain messenger ribonucleic acids (mRNAs) that code for neurotransmitter receptors, and that these messengers can be translated in *Xenopus* oocytes to cause the formation of functional receptors in the oocyte membrane. By using this approach, brain mRNA has been shown to induce the oocytes to acquire sensitivity to 'excitatory' and 'inhibitory' amino acids (Miledi *et al.* 1982; Gundersen *et al.* 1984*a*–*c*), serotonin (Gundersen *et al.* 1983, 1984*c*; Parker *et al.* 1984) acetylcholine (Sumikawa *et al.* 1984*b*), dopamine and noradrenaline (Sumikawa *et al.* 1984*c*), and neuropeptides (Parker *et al.* 1986*b*). This 'transplantation' of brain receptors into a more amenable cell offers many advantages for electrophysiological study, and the finding that mRNAs coding for different receptors may be partly separated by sucrose density centrifugation (Sumikawa *et al.* 1984*a*, *b*) further extends the possibilities. Here, we have used these techniques to study brain receptors activated by neutral amino acids.

The neutral amino acids glycine, γ-aminobutyric acid (GABA) and β-alanine all have inhibitory effects when applied to neurons in the central nervous system of vertebrates (for a recent review see Snodgrass (1983)). However, although it is well established that GABA and glycine act on different receptors, it is not yet clear whether β-alanine acts on a specific receptor, or whether it activates GABA or glycine receptors (Nistri & Constanti 1979; Barker *et al.* 1982; Snodgrass 1983). We have examined this question by measuring the responses to β-alanine, glycine and GABA induced in oocytes by different fractions of mRNA from rat brain, and by mRNAs from different sources. The relative sizes of the responses to GABA and glycine induced by different messenger preparations differ widely (Sumikawa *et al.* 1984*b*), presumably because different quantities of functional receptors are inserted in the oocyte membrane. If the responses to β-alanine arose because of activation of glycine or GABA receptors, then the sensitivity to β-alanine induced by different mRNAs would be expected to parallel that to glycine or GABA. Our results show that this is not the case, and instead, suggest that there may be a distinct receptor for β-alanine.

Methods

Experiments were made on oocytes of *Xenopus laevis*, which were injected with whole poly $(A)^+$ mRNA derived from chick optic lobe and brain (Miledi *et al.* 1982), from rat brain and cerebral cortex (Gundersen *et al.* 1984*a*), or with fractions of mRNA obtained after sucrose density gradient centrifugation of the mRNA from these sources (Sumikawa *et al.* 1984*b*). Methods for the isolation and injection of mRNA, and for electrophysiological recording from oocytes were as described previously (Miledi & Sumikawa 1982; Miledi 1982; Kusano *et al.* 1982). Techniques for size fractionation of mRNA were also as used previously (Sumikawa *et al.* 1984*a*, *b*), and the fractions of rat brain and chick optic lobe mRNA used in the present work correspond to those in Sumikawa *et al.* (1984*b*). Oocytes were usually treated with collagenase to remove follicular cells and thus avoid complications arising from the presence of these and other enveloping cells (Miledi & Parker 1984). Recordings were made of membrane currents elicited by bath application of drugs to oocytes continuously superfused with normal Ringer solution (composition (mM): NaCl, 120; KCl, 2; $CaCl_2$, 1.8; HEPES, 5; pH 7.2), at a temperature of about 20 °C. Unless otherwise stated, all records were obtained with the oocyte membrane voltage clamped at a potential of −60 mV. Many of the experiments described here were done at University College London, in 1984.

Results

Responses to neutral amino acids induced by brain mRNA

Native (non-injected) oocytes of *Xenopus laevis* show either no responses to high (1 mM) concentrations of glycine and GABA, or give inward currents that are just a few nanoamperes in amplitude (Kusano *et al.* 1982; Miledi *et al.* 1982; Gundersen *et al.* 1984*b*). In contrast to this, oocytes injected with mRNA derived from various regions of rat and chick brains showed large membrane responses to one or both of these amino acids (figure 1 and table 1; and see Miledi *et al.* (1982); Gundersen

et al. (1984*b*); Houamed *et al.* (1984)) due to activation of exogenous receptor-channel complexes translated from the injected mRNA (Gundersen *et al.* 1984*a*, *b*). The amino acids elicited smooth, desensitizing membrane currents, which were inward at a potential of −60 mV. As described before (Miledi *et al.* 1982; Gundersen *et al.* 1984*b*), these currents invert direction at about the chloride equilibrium potential of the oocyte, and are both carried largely by chloride ions. We did not observe responses to baclofen (1 mM) in oocytes injected with either chick optic lobe or rat brain mRNA, even when GABA elicited large responses. This, together with the ionic specificity and pharmacological properties of the GABA responses (see later), suggests that the receptors induced in the oocyte were of the $GABA_A$ type (Hill & Bowery 1981).

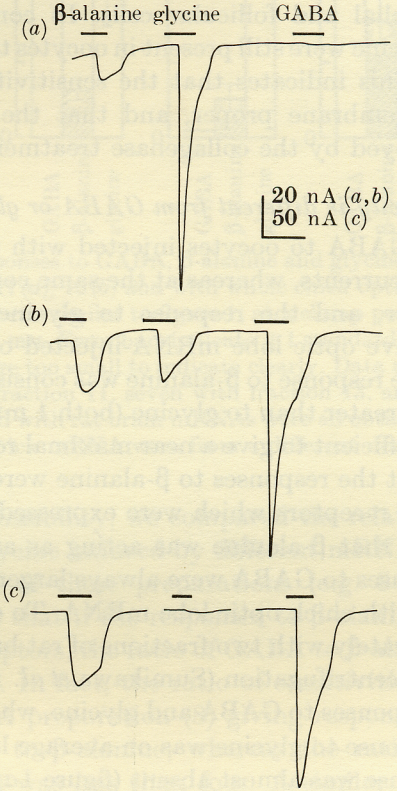

FIGURE 1. Membrane currents elicited by neutral amino acids in *Xenopus* oocytes injected with brain mRNAs. Each block shows responses to (from left to right) β-alanine (1 mM), glycine (1 mM) and GABA (1 mM), applied by bath perfusion as indicated by the bars. Oocytes were voltage clamped at a potential of −60 mV, and in this and other figures, downward deflections correspond to inward membrane currents. The oocytes were injected with rat brain mRNA fractions 13 (*a*) and 11 (*b*), and with whole chick optic lobe mRNA (*c*).

In oocytes injected with chick optic lobe or rat brain mRNA, β-alanine also elicited smooth inward membrane currents, similar to the GABA and glycine currents (figure 1). The sensitivity to β-alanine developed as a result of the injection of exogenous mRNA, and was not present in non-injected oocytes in the

present experiments, although oocytes from some donors show small (a few nanoamperes) native responses to β-alanine. For example, three control oocytes from the same donor as illustrated in figures 1*a*, *b* and 2*a*, *b* failed to give detectable responses to β-alanine (1 mM), and four other oocytes injected with a relatively ineffective fraction of rat brain mRNA (fraction 4; see Sumikawa *et al.* 1984*b*) gave a mean response of only 2.5 nA. The responses to GABA, glycine and β-alanine all showed a marked desensitization when the agonists were applied at a concentration of 1 mM. As a result of this, measurements of the peak response sizes are probably underestimated. None the less, we used similar conditions for all oocytes, so that comparisons between oocytes should remain valid.

Kusano *et al.* (1982) have shown that the responses of native oocytes to catecholamines disappear after the oocytes are treated with collagenase so as to remove the inner epithelial and follicular cells. In contrast, the responses to GABA, β-alanine and glycine were still present in oocytes that had been collagenase treated (e.g. figure 1). This indicates that the sensitivity to these amino acids resides in the oocyte membrane proper, and that the induced receptors and channels were not destroyed by the collagenase treatment.

β-alanine sensitivity is different from GABA or glycine sensitivity

Application of 1 mM GABA to oocytes injected with chick optic lobe mRNA elicited large membrane currents, whereas at the same concentration the response to β-alanine was smaller, and the response to glycine was barely detectable (figures 1*c* and 2*c*). In five optic lobe mRNA-injected oocytes tested with both β-alanine and glycine, the response to β-alanine was consistently larger and was on average about 30 times greater than to glycine (both 1 mM). Because a concentration of 1 mM glycine is sufficient to give a near maximal response (Gundersen *et al.* 1984*b*), this suggests that the responses to β-alanine were presumably not due to activation of the glycine receptors which were expressed by the chick mRNA.

It could be, however, that β-alanine was acting as an agonist at the GABA receptor, since the responses to GABA were always larger than those to β-alanine in the oocytes injected with chick optic lobe mRNA. To examine this possibility, we injected oocytes separately with two fractions of rat brain mRNA prepared by sucrose density gradient centrifugation (Sumikawa *et al.* 1984*b*). Oocytes injected with fraction 11 gave responses to GABA and glycine, whereas in oocytes injected with fraction 13 the response to glycine was on average larger than with fraction 11, and the GABA response was almost absent (figure 1*a*, *b* and 2*a*, *b*). In spite of the thirtyfold difference in GABA sensitivity induced by these two mRNA fractions, the mean response to β-alanine was about the same (figure 2*a*, *b*). This suggests that the responses to β-alanine did not arise because of activation of GABA receptors alone. Further evidence supporting this conclusion is that the seven oocytes injected with rat brain mRNA fraction 13 all showed larger responses to 1 mM β-alanine than to 1 mM GABA (e.g. figure 1*a*), even though this concentration of GABA is sufficient to give a maximal response (figure 4).

The results above clearly indicate that the response to β-alanine could not have been due to β-alanine acting exclusively on GABA or glycine receptors. However, it could be that β-alanine acts as a partial agonist on both GABA and glycine

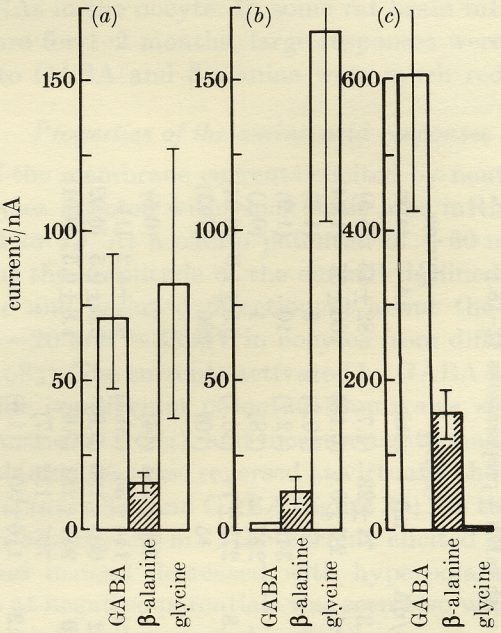

FIGURE 2. Mean sizes of responses to GABA, β-alanine and glycine in oocytes injected with rat brain mRNA fractions 11 (*a*), 13 (*b*) and with whole chick optic lobe mRNA (*c*). Responses were measured from records similar to figure 1, at a clamp potential of −60 mV, and with drug concentrations of 1 mM. Error bars indicate ±1 standard error of the mean, and where not shown the errors were too small to indicate clearly. Data are from five oocytes injected with rat brain mRNA fraction 11, seven with fraction 13, and five with chick optic lobe mRNA. Oocytes injected with rat brain mRNAs were all obtained from a single donor, and oocytes injected with chick mRNA were from a different donor.

receptors. To test this possibility, we compared the relative sizes of responses to β-alanine, GABA and glycine induced by different mRNA preparations and fractions (table 1.) Several of these preparations (e.g. 8–18) induced almost no sensitivity to glycine, so that if the responses to β-alanine had arisen because of activation of GABA receptors, the ratio of GABA to β-alanine responses would be expected to be constant. In fact, the ratio of sensitivities varied nearly tenfold, with a rat cortex mRNA preparation (8) giving responses to GABA more than forty times larger than to β-alanine, whereas the ratio with chick optic lobe mRNA (preparation 10) was less than four. A similar variation was seen with different fractions of chick optic lobe mRNA, which induced virtually no sensitivity to glycine. For example, chick optic lobe mRNA fraction 10 induced responses to GABA that were on average 30 times greater than those to β-alanine (four oocytes), whereas the corresponding ratio for fraction 15 mRNA was 11.1 (five oocytes).

Another argument is that with some mRNA preparations the responses to β-alanine were small, despite the presence of large responses to GABA or glycine (e.g. preparations 2 and 8, table 1). Activation of GABA or glycine receptors by β-alanine in these preparations was therefore negligible. Finally, it appeared that there were differences in the stabilities of the amino acid receptors and their

TABLE 1. SIZES OF MEMBRANE CURRENTS ELICITED BY β-ALANINE, GABA AND GLYCINE IN OOCYTES INJECTED WITH DIFFERENT PREPARATIONS OF mRNA

(Peak sizes of responses were recorded at a clamp potential of −60 mV, with agonist concentrations of 1 mM. Figures give mean ±1 standard error of the mean, and numbers in brackets indicate the response sizes to GABA and glycine as a fraction of that to β-alanine. Rat and chick fractions correspond to those given in Sumikawa *et al.* (1984*b*). The total poly(A)$^+$ mRNA preparations were different from those used to derive the fractions. Responses to glycine were negligible (2 nA or less) in oocytes injected with mRNA preparations 12–18.)

preparation no	mRNA type	no. of oocytes	β-alanine/nA	GABA nA	GABA ratio	glycine nA	glycine ratio
1	rat brain fraction 11	5	15±3.3	71±23	(4.7)	82±45	(5.5)
2	rat brain fraction 13	7	12.4±4.4	2.3±0.6	(0.18)	166±57	(13.4)
3	rat cerebral cortex fraction 7	3	4	41	(10.2)	5	(1.2)
4	rat cerebral cortex fraction 9	3	8	342	(42.7)	10	(1.2)
5	rat cerebral cortex fraction 11	3	8	227	(28)	1	(0.1)
6	rat cerebral cortex	9	7.4±1.8	272±74	(36.7)	2.8±1.0	(0.34)
7	rat cerebral cortex after 2 months in culture	2	1.5	15	(10)	290	(193)
8	rat cerebral cortex	7	13.4±3	590±154	(44)	7	(0.5)
9	rat whole brain	6	5.5±1.1	114±33	(20.7)	6.0±4.2	(1)
10	chick optic lobe	5	154±32	603±180	(3.9)	3.0±0.5	(0.02)
11	chick optic lobe	9	124±49	947±281	(7.6)	1.8±0.5	(0.01)
12	chick optic lobe fraction 12	7	47±6	797±137	(17)		
13	chick optic lobe fraction 15	5	10±2.3	116±35	(11.6)		
14	chick cerebral cortex	9	70.1±10.7	379±53	(5.4)		
15	chick cerebellum	11	18.6±3.6	115±16	(6.1)		
16	chick optic lobe	10	77.5±10.1	653±97	(8.4)		
17	chick cerebral cortex	9	70.1±10.7	379±53	(5.4)		
18	chick cerebellum	11	18.6±3.6	115±16	(6.1)		

corresponding mRNAs in the oocyte. In some rat brain mRNA-injected oocytes maintained in culture for 1–2 months, large responses were obtained to glycine, but the responses to GABA and β-alanine were much reduced (preparation 7, table 1).

Properties of the amino acid responses

The properties of the membrane currents elicited by neutral amino acids were investigated in oocytes injected with chick optic lobe mRNA, or with rat brain mRNA fractions 11 or 13. At a clamp potential of −60 mV, β-alanine elicited inward currents, but the amplitude of the current declined as the potential was made more positive and inverted direction at about the chloride equilibrium potential (between −20 and −35 mV in oocytes from different donors; Kusano *et al.* 1982; Barish 1983). The currents activated by GABA and glycine also invert at about the chloride equilibrium potential (Sumikawa *et al.* 1982; Gundersen *et al.* 1984*b*; Houamed *et al.* 1984), and measurements made on the same oocyte showed that the β-alanine response reversed at virtually the same potential as the responses to glycine (figure 3*a*) and GABA (figure 3*b*). As the potential was made more negative than about −80 mV, the current elicited by β-alanine failed to increase further, and instead decreased with hyperpolarization (figure 3*b*). A similar rectification at negative potentials was seen also with responses to GABA (figure 3*b*) and glycine (Gundersen *et al.* 1984*b*).

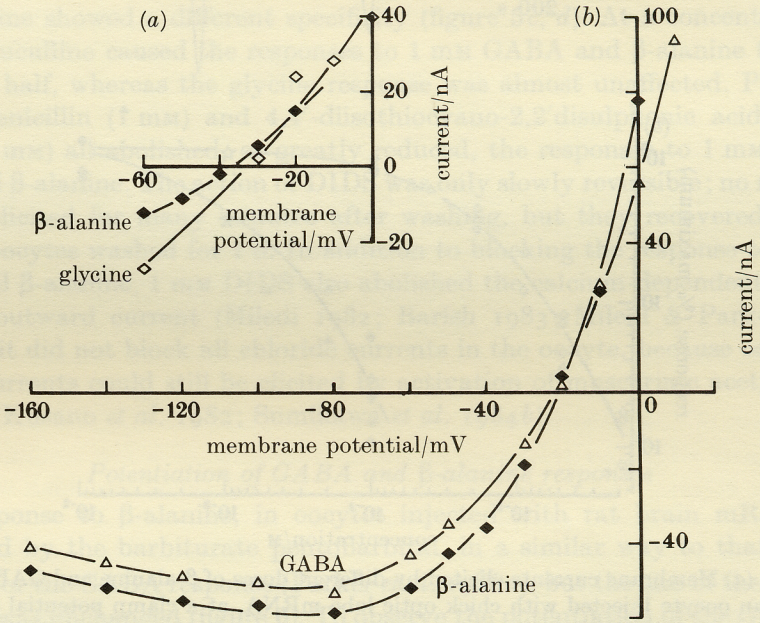

FIGURE 3. Current-voltage relations for the currents activated by β-alanine (◆), GABA (△) and glycine (◇). Measurements were obtained by briefly stepping the potential to different values from a holding potential of −60 mV, before applying drugs and then repeating in the continued presence of drugs (both 1 mM). Points show the agonist-activated currents at each potential, after subtraction of 'passive' currents. (*a*) Currents elicited by glycine (1 mM) and β-alanine (1 mM) measured in an oocyte injected with rat brain mRNA fraction 11. (*b*) Currents elicited by GABA (10 μM) and β-alanine (1 mM) in an oocyte injected with whole chick optic lobe mRNA.

Figure 4*a* illustrates membrane currents elicited by different concentrations of β-alanine applied to an oocyte injected with chick optic lobe mRNA. Responses first became detectable at a concentration of about 10^{-4} M, and increased as the concentration was raised to 10^{-2} M. At low doses, a doubling in concentration gave a more than twofold increase in response (e.g. first two traces in figure 4*a*). Similar to the currents elicited by GABA (Miledi *et al.* 1982; Parker *et al.* 1986*a*) and glycine (Gundersen *et al.* 1984*b*), the responses to β-alanine were well maintained at low doses, but showed a decline during the application of higher doses of agonist (figure 4*a*). However, at equimolar concentrations, the responses to β-alanine desensitized more slowly than those to GABA or glycine. The concentration dependence of the peak currents elicited by GABA and β-alanine is shown in figure 4*b*, measured in oocytes injected with chick optic lobe mRNA or rat brain mRNA. The data obtained with these two preparations of mRNA were similar

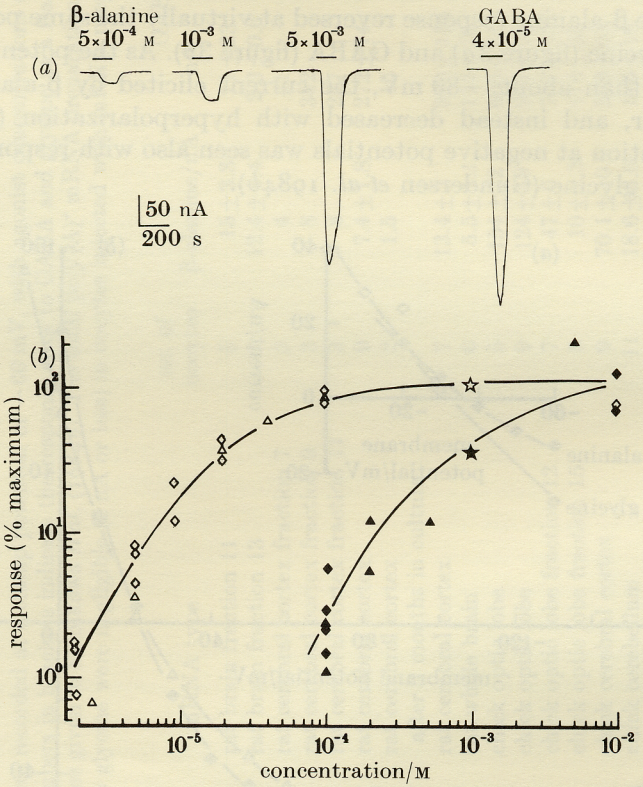

FIGURE 4. (*a*) Membrane currents elicited by different doses of β-alanine and GABA, recorded from an oocyte injected with chick optic lobe mRNA, at a clamp potential of −60 mV. Drugs were applied by bath perfusion, for the times indicated by the bars. (*b*) Dose–response for membrane currents elicited by GABA (open symbols) and β-alanine (filled symbols). Points marked by diamonds were obtained in oocytes injected with whole rat brain mRNA, and triangles in oocytes injected with whole chick optic lobe mRNA. For both amino acids the measurements at different concentrations were normalized with respect to the response obtained in each oocyte at a concentration of 1 mM (star), and are plotted as a percentage of the mean maximum size. Data are from six oocytes (three rat and three chick) for β-alanine, and three oocytes (two rat and one chick) for GABA.

for both amino acids. However, GABA was clearly effective at much lower concentrations that β-alanine; half-maximal activation was obtained with concentrations of *ca.* 3×10^{-5} M GABA and 10^{-3} M β-alanine. At low doses, the slope of the dose–response curve for GABA was about 1.7 on double logarithmic scales (figure 4), suggesting that at least two molecules of GABA are required to cause channel opening (see also Miledi *et al.* 1982; Parker *et al.* 1986*a*). The data for β-alanine also fitted reasonably well to the same slope value (figure 4). Different to this, the concentration dependence of the glycine response in oocytes injected with human fetal brain mRNA is much steeper, with a limiting slope value of 2.7 (Gundersen *et al.* 1984*b*). Glycine responses in oocytes injected with rat brain mRNA also showed a steep concentration dependence, and five oocytes that were each tested with two concentrations in the range 10^{-4} to 3×10^{-4} M gave a mean slope of 2.6 (range 2–3.5).

Blocking agents on amino acid responses

Strychnine (10 μM) abolished almost completely the responses to β-alanine and glycine (both 1 mM), but had little effect on the response to 1 mM GABA (figure 5*a*, *b*). Even at a concentration of 1 mM, strychnine failed to reduce the response to 1 mM GABA appreciably, although with lower concentrations of GABA such a reduction was observed (cf. Miledi *et al.* 1982). For example, 10 μM strychnine lowered the response elicited by 10 μM GABA to about one tenth.

Bicuculline showed a different specificity (figure 5*c*, *d*). At a concentration of 140 μM bicuculline caused the responses to 1 mM GABA and β-alanine to fall by about one half, whereas the glycine response was almost unaffected. Picrotoxin (20 μM), penicillin (1 mM) and 4,4′-diisothiocyano-2,2′disulphonic acid stilbene (DIDS) (1 mM) all abolished, or greatly reduced, the responses to 1 mM glycine, GABA and β-alanine. The action of DIDS was only slowly reversible; no responses could be elicited for many minutes after washing, but they recovered at least partly in oocytes washed for 1 d. In addition to blocking the response to GABA, glycine and β-alanine, 1 mM DIDS also abolished the calcium-dependent chloride transient outward current (Miledi 1982; Barish 1983; Miledi & Parker 1984). However, it did not block all chloride currents in the oocyte, because oscillatory chloride currents could still be elicited by activation of muscarinic acetylcholine receptors (Kusano *et al.* 1982; Sumikawa *et al.* 1984*b*).

Potentiation of GABA and β*-alanine responses*

The response to β-alanine, in oocytes injected with rat brain mRNA, was potentiated by the barbiturate pentobarbital, in a similar way to that already described for the GABA response (Parker *et al.* 1986*a*), but the size of the response to glycine was unchanged (figure 6). To observe the potentiation of the GABA and β-alanine responses, it was necessary to use relatively low concentrations of amino acids, which gave less than maximal activation (cf. figure 4). Little potentiation was seen, for example, with 1 mM GABA. Measurements from three oocytes indicated that the size of the glycine response was unchanged by 100 μM pentobarbital (mean size 98% control), but that the GABA response increased by a factor of about four, and the β-alanine response by a factor of more than twenty.

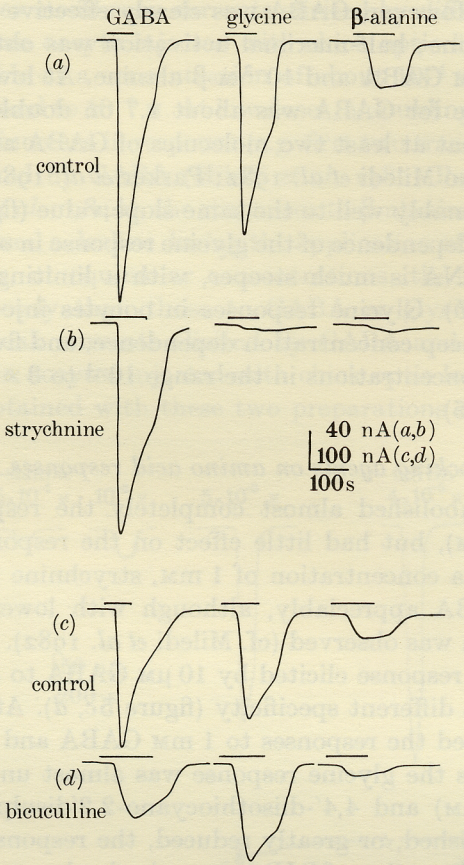

FIGURE 5. Blocking actions of strychnine (*a*, *b*) and bicuculline (*c*, *d*) on the responses to GABA (left), glycine (middle) and β-alanine (right). All records were obtained at a clamp potential of −60 mV and with amino acid concentrations of 1 mM. (*a*, *b*) Responses from an oocyte injected with rat brain mRNA fraction 11, recorded in normal Ringer (*a*), and in the continued presence of strychnine (*b*). Perfusion with strychnine (10 μM) began a few minutes before the first record in (*b*) was obtained. (*c*, *d*) Similar records from a different oocyte, also injected with rat brain mRNA fraction 11, showing the effect of bicuculline (140 μM).

Application of 100 μM pentobarbital, by itself, to oocytes injected with rat brain mRNA gave either no response, or inward currents of a few nanoamperes (at −60 mV). Higher concentrations gave larger inward currents, which were associated with a decrease in membrane resistance.

The benzodiazepines flunitrazepam and chlorazepate also potentiated the responses to GABA and β-alanine, without enhancing the response to glycine (figure 7). The concentrations of benzodiazepines used in figure 7 were sufficient to give a maximal increase of the GABA response. Even so, potentiation of the GABA and β-alanine responses was less than with barbiturate. Mean values of the GABA response were 180% of the control (five oocytes, pooling data with both 1 μM flunitrazepam and 10 μM chlorazepate), and the β-alanine response was potentiated to 200% of the control (three oocytes). Curiously, the current elicited by glycine showed a more rapid desensitization in the presence of chlorazepate,

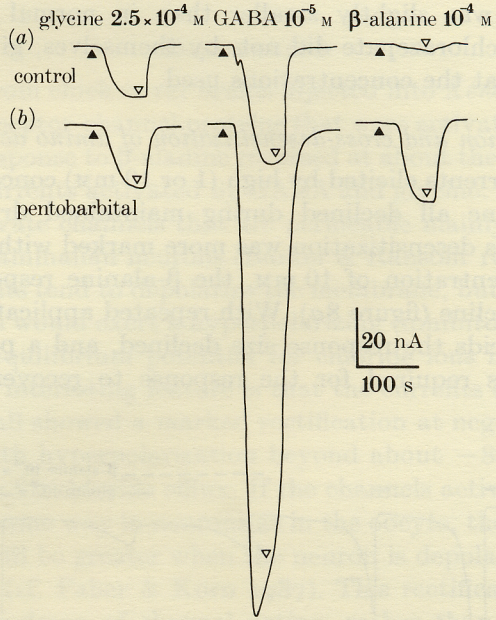

FIGURE 6. Potentiating action of pentobarbital on the responses to GABA and β-alanine, but not to glycine. Amino acids were applied at the concentrations indicated, with the oocyte in normal Ringer solution (*a*), and then in the continued presence of 100 μM pentobarbital (*b*), beginning a few minutes before the first record. Oocyte was injected with rat brain mRNA fraction 11.

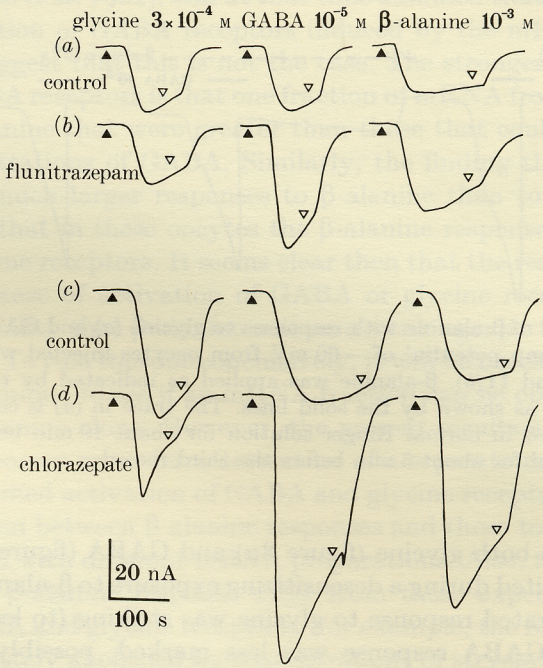

FIGURE 7. Effect of benzodiazepines on the responses to glycine (left), GABA (middle) and β-alanine (right). Amino acid concentrations in all records were as indicated, and the clamp potential was −60 mV. (*a*, *b*) Records from an oocyte injected with rat brain mRNA fraction 11, obtained in normal Ringer solution (*a*), and in the continued presence of 1 M flunitrazepam (*b*). (*c*, *d*) Similar records from a different oocyte, also injected with fraction 11 mRNA, showing the effect of chlorazepate (10 μM).

and the peak size was slightly smaller than in normal Ringer (figure 7*d*). Flunitrazepam and chlorazepate did not, by themselves, give rise to detectable membrane currents at the concentrations used.

Desensitization and cross-desensitization of amino acid responses

The membrane currents elicited by high (1 or 10 mM) concentrations of GABA, glycine and β-alanine all declined during maintained drug application (e.g. figures 1 and 8.) This desensitization was more marked with GABA and glycine, and even at a concentration of 10 mM, the β-alanine response showed a comparatively slower decline (figure 8*a*). With repeated applications of high concentrations of amino acids the response size declined, and a period of washing for several minutes was required for the response to recover to the initial size (figure 8*a*).

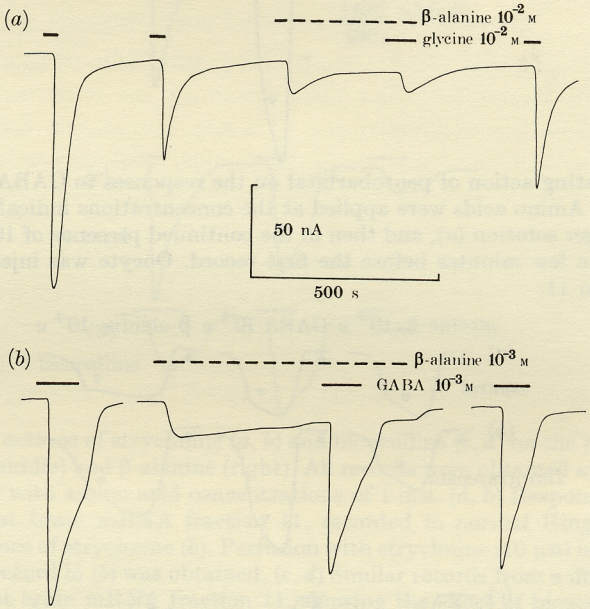

FIGURE 8. Interaction of β-alanine with responses to glycine (*a*) and GABA (*b*). Records were obtained at a clamp potential of −60 mV from oocytes injected with rat brain mRNA fractions 13(*a*) and 11(*b*). β-alanine was applied as indicated by the broken lines, and glycine or GABA as shown by the solid lines. The trace in (*a*) is continuous. In (*b*), the oocyte was washed in normal Ringer solution for about 10 min before the start of the second record, and for about 5 min before the third record.

The responses to both glycine (figure 8*a*) and GABA (figure 8*b*) were reduced when they were elicited during a desensitizing exposure to β-alanine. The reduction in size of the illustrated response to glycine was striking (to less than 15%), but the effect on the GABA response was less marked, possibly because a lower concentration of β-alanine was used in this instance.

Discussion

Messenger RNA from chick or rat brains injected into *Xenopus* oocytes induced the appearance of receptor-channel proteins that were activated by β-alanine. The current flowing in response to β-alanine reversed at about the chloride equilibrium potential, like the currents activated by GABA and glycine. Thus all three amino acids appear to activate channels that are permeable mainly to chloride ions, as is the case also in mammalian neurons (Barker & Ransom 1978*a*). In the oocyte, these chloride currents tend to depolarize the membrane, but in neuronal cells the amino acid receptors would exert a hyperpolarizing (inhibitory) effect, because of the more negative equilibrium potential for chloride ions in neurons compared with the oocyte. An interesting feature is that the currents elicited by β-alanine, GABA and glycine all showed a marked rectification at negative potentials, and failed to increase with hyperpolarization beyond about −80 mV despite the increased driving force for chloride efflux. If the channels activated by these amino acids behave in the same way in neurons as in the oocyte, the inhibitory action of these transmitters will be greater when the neuron is depolarized than when it is at resting potential (cf. Faber & Korn 1987). This rectification probably arises from a voltage-dependence of channel gating, rather than a decrease in single channel conductance (Gundersen *et al.* 1986; Borman *et al.* 1987; Faber & Korn 1987).

We first observed responses to β-alanine in oocytes injected with chick optic lobe mRNA (Miledi *et al.* 1982), and at that time assumed that the responses arose because of activation of GABA receptors induced by the mRNA. However, the present results suggest that this is not the case. The strongest argument against activation of GABA receptors is that one fraction of mRNA from rat brain induced responses to β-alanine that were greater than those that could be obtained with even high concentrations of GABA. Similarly, the finding that chick optic lobe mRNA induced much larger responses to β-alanine than to glycine provides a strong argument that in these oocytes the β-alanine response did not arise from activation of glycine receptors. It seems clear then that the responses to β-alanine did not arise because of activation of GABA or glycine receptors alone. There remains the possibility that β-alanine acts as a weak agonist for both GABA and glycine receptors. To rule this out conclusively, it will be necessary to improve the fractionation technique to see if an mRNA fraction can be obtained that induces sensitivity to β-alanine alone. However, the present results already suggest that the β-alanine responses arose from activation of a separate receptor, rather than because of a combined activation of GABA and glycine receptors. Thus, there was a lack of correlation between β-alanine responses and those to GABA and glycine in oocytes injected with different mRNA preparations. Also, the pharmacological properties of the β-alanine response differ from those expected of a combined activation of GABA and glycine receptors. For example, the response to β-alanine was almost completely abolished by concentrations of strychnine which had little effect on the GABA response, suggesting that activation of GABA receptors could have contributed little to the β-alanine response, and yet the response to β-alanine was potentiated by barbiturate and reduced by bicuculline in a similar fashion to the GABA response.

Thus, the mRNA from chick and rat brains appears to contain distinct translationally active messengers coding for receptors to GABA and glycine, and probably also for β-alanine. Presumably, these messengers arose in the main from those neuronal cells, in which they normally lead to the expression of receptors similar to those induced here in the oocyte. However, we cannot rule out other cellular sources; for example, there is evidence that astrocytes show responses to excitatory (though not inhibitory) amino acids (Bowman & Kimelberg 1984).

The pharmacological properties of the GABA receptor expressed in the oocyte resemble those of $GABA_A$ receptors in vertebrate central neurons; the responses were antagonized by picrotoxin, bicuculline and penicillin, but not by strychnine at doses that blocked the glycine response (Curtis & Johnson 1974; Nistri & Constanti 1979), and they were potentiated by barbiturate (Barker & Ransom 1978*b*) and benzodiazepines (Haefely *et al.* 1979). The response to β-alanine showed similar pharmacological properties to GABA, but with the important differences that the response was nearly abolished by concentrations of strychnine which had little effect on the GABA response. This antagonism of the actions of β-alanine is also seen in the brain, and has sometimes led to the classification of β-alanine as a 'glycine like' amino acid (Nistri & Constanti 1979), although its antagonism by bicuculline has also been observed in neurons (Curtis *et al.* 1971).

The findings that barbiturates and benzodiazepines potentiate the response of a specific receptor for β-alanine may be of importance in the clinical actions of these drugs, in addition to their well-known potentiation of GABA responses (Barker & Ranson 1978*b*; Haefely *et al.* 1979; Braestrup & Nielsen 1983). Potentiation of responses to β-alanine by barbiturate has previously been seen in frog sensory neurons (Akaike *et al.* 1985), but in contrast to this, Barker & Ransom (1978*b*) reported that pentobarbitone did not enhance β-alanine responses in cultured mammalian spinal cord neurons, even though GABA responses in the same cells were potentiated. Explanations for these differences may include the possibility of different types of β-alanine receptors in different neurons, or variations in the ability of β-alanine to activate different GABA receptors.

Sustained application of β-alanine induced current responses that desensitized over a period of a few minutes. The responses to glycine and GABA, applied at this time in addition to the β-alanine, were reduced compared with control responses without β-alanine. A similar mutual antagonism between inhibitory amino acids has also been seen in cultured neurons (Barker & McBurney 1979; Barker *et al.* 1982). Such 'cross desensitization' could be most easily explained if the different agonists acted at the same receptor site, but we regard this as unlikely because of the strong evidence for separate β-alanine receptors. Also, mutual antagonism has been shown in spinal motorneurons between glycine and GABA, even though these two amino acids almost certainly activate different receptors (Barker & McBurney 1979). Possible mechanisms for the antagonism between amino acids have been discussed by Barker *et al.* (1982).

Our previous results with mRNA fractionation indicated that GABA and glycine receptors are encoded by different species of mRNA (Sumikawa *et al.* 1984*b*). The genes encoding subunits of these receptors have recently been cloned, and their nucleotide sequences were found to show a large degree of homology

(Schofield *et al.* 1987; Grenningloh *et al.* 1987). The data presented here suggest that a receptor for β-alanine is coded by a distinct mRNA species, and because of the functional similarities in responses to β-alanine, glycine and GABA, it would not be surprising if this receptor also showed many structural homologies.

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References

Akaike, N., Hattori, K., Inomata, N. & Oomura, Y. 1985 γ-aminobutyric acid- and pentobarbitone-gated chloride currents in internally perfused frog sensory neurones. *J. Physiol., Lond.* **360**, 367–386.

Barish, M. E. 1983 A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol., Lond.* **342**, 309–325.

Barker, J. L. & McBurney, R. N. 1979 GABA and glycine may share the same conductance channel on cultured mammalian neurones. *Nature, Lond.* **277**, 234–236.

Barker, J. L., McBurney, R. N. & Macdonald, J. F. 1982 Fluctuation analysis of neutral amino acid responses in cultured mouse spinal motoneurones. *J. Physiol., Lond.* **322**, 365–387.

Barker, J. L. & Ransom, B. R. 1978*a* Amino acid pharmacology of mammalian central neurones grown in tissue culture. *J. Physiol., Lond.* **280**, 331–354.

Barker, J. L. & Ransom, B. R. 1978*b* Pentobarbitone pharmacology of mammalian central neurones grown in tissue cultures. *J. Physiol., Lond.* **280**, 355–372.

Bormann, J., Hamill, O. P. & Sakmann, B. 1987 Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurones. *J. Physiol., Lond.* **383**, 243–286.

Bowman, C. L. & Kimelberg, H. K. 1984 Excitatory amino acids directly depolarize rat brain astrocytes in primary culture. *Nature, Lond.* **311**, 656–659.

Braestrup, C. & Nielsen, M. 1983 Benzodiazepine Receptors. In *Handbook of psychopharmacology*, vol. 17 (*Biochemical studies of CNS receptors*) (ed. L. L. Iversen, S. D. Iversen & S. H. Snyder), pp. 285–384. New York: Plenum Press.

Curtis, D. R., Duggan, A. W., Felix, D., Johnston, G. A. R. & McLennan, H. 1971 Antagonism between bicuculline and GABA in the cat brain. *Brain Res.* **33**, 57–73.

Curtis, D. R. & Johnston, G. A. R. 1974 Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.* **69**, 97–188.

Faber, D. S. & Korn, H. 1987 Voltage-dependence of glycine-activated Cl^- channels: a potentiometer for inhibition? *J. Neurosci.* **7**, 807–811.

Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E. D. & Betz, H. 1987 The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. *Nature, Lond.* **328**, 215–220.

Gundersen, C. B., Miledi, R. & Parker, I. 1983 Serotonin receptors induced by exogenous messenger RNA in *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **219**, 103–109.

Gundersen, C. B., Miledi, R. & Parker, I. 1984*a* Glutamate and kainate receptors induced by rat brain messenger RNA in *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **221**, 127–143.

Gundersen, C. B., Miledi, R. & Parker, I. 1984*b* Properties of human brain glycine receptors expressed in *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **221**, 235–244.

Gundersen, C. B., Miledi, R. & Parker, I. 1984*c* Messenger RNA from human brain induces drug- and voltage-operated channels in *Xenopus* oocytes. *Nature, Lond.* **308**, 421–424.

Gundersen, C. B., Miledi, R. & Parker, I. 1986 Voltage dependence of human brain glycine receptor-channels in *Xenopus* oocytes. *J. Physiol., Lond.* **377**, 40*P*.

Haefely, W., Polc, P., Schaffner, R., Keller, H. H., Pieri, L. & Moehler, H. 1979 Facilitation of GABAergic transmission by drugs. In *GABA-neurotransmitters* (ed. P. Krogsgaard-Larsen, J. Scheel-Kruger & H. Kofod), pp. 357–375. New York: Academic Press.

Hill, D. R. & Bowery, N. G. 1981 [^{3}H]Baclofen and [^{3}H]GABA binding to bicuculline insensitive GABA sites in rat brain. *Nature, Lond.* **290**, 149–152.

Houamed, K. M., Bilbe, G., Smart, T. G., Constanti, A., Brown, D. A., Barnard, E. A. & Richards, B. M. 1984 Functional expression of GABA, glycine and glutamate receptors in *Xenopus* oocytes by injection of rat brain mRNA. *Nature, Lond.* **310**, 318–321.

Kusano, K., Miledi, R. & Stinnakre, J. 1982 Cholinergic and catecholaminergic receptors in the *Xenopus* oocyte membrane. *J. Physiol., Lond.* **328**, 143–179.

Miledi, R. 1982 A calcium-dependent transient outward current in *Xenopus laevis* oocytes. *Proc. R. Soc. Lond.* B **215**, 491–494.

Miledi, R. & Parker, I. 1984 Chloride current induced by injection of calcium into *Xenopus* oocytes. *J. Physiol., Lond.* **357**, 173–183.

Miledi, R., Parker, I. & Sumikawa, K. 1982 Synthesis of chick brain GABA receptors by frog oocytes. *Proc. R. Soc. Lond.* B **216**, 509–515.

Miledi, R. & Sumikawa, K. 1982 Synthesis of cat muscle acetylcholine receptors by *Xenopus* oocytes. *Biomed. Res.* **3**, 390–399.

Nistri, A. & Constanti, A. 1979 Pharmacological characterization of different types of GABA and glutamate receptors in vertebrates and invertebrates. *Prog. Neurobiol.* **13**, 117–235.

Parker, I., Gundersen, C. B. & Miledi, R. 1984 A transient inward current elicited by hyperpolarization during serotonin activation in *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **223**, 279–292.

Parker, I., Gundersen, C. B. & Miledi, R. 1986*a* Actions of pentobarbital on rat brain receptors expressed in *Xenopus* oocytes. *J. Neurosci.* **6**, 2290–2297.

Parker, I., Sumikawa, K. & Miledi, R. 1986*b* Neurotensin and substance P receptors expressed in *Xenopus* oocytes by messenger RNA from rat brain. *Proc. R. Soc. Lond.* B **229**, 151–159.

Schofield, P. R., Darlison, M. G., Fijita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Real, V., Glencosse, T. A., Seeburg, P. H. & Barnard, E. A. 1987 Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor superfamily. *Nature, Lond.* **328**, 221–227.

Snodgrass, S. R. 1983 Receptors for amino acid transmitters. In *Handbook of pharmacology* vol. 17 (*Biochemical studies of CNS receptors*) (ed. L. L. Iversen, S. D. Iversen & S. H. Snyder), pp. 167–239. New York: Plenum Press.

Sumikawa, K., Parker, I., Amano, T. & Miledi, R. 1984*a* Separate fractions of mRNA from *Torpedo* electric organ induce chloride channels and acetylcholine receptors in *Xenopus* oocytes. *EMBO J.* **3**, 2291–2294.

Sumikawa, K., Parker, I. & Miledi, R. 1984*b* Partial purification and functional expression of brain mRNAs coding for neurotransmitter receptors and voltage-operated channels. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7994–7998.

Sumikawa, K., Parker, I. & Miledi, R. 1984*c* Messenger RNA from rat brain induces noradrenaline and dopamine receptors in *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **223**, 255–260.