Proc. R. Soc. Lond. B **216**, 509–515 (1982)
Printed in Great Britain

Synthesis of chick brain GABA receptors by frog oocytes

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(*Received* 10 *September* 1982)

Poly(A)-mRNA, extracted from the optic lobe of chick embryos, directs the synthesis of γ-aminobutyric acid (GABA) receptors in *Xenopus laevis* oocytes. The receptors are inserted into the oocyte membrane, where they form receptor–channel complexes. When activated by GABA, and related agonists, the chick brain receptors open membrane channels that are permeable to chloride ions. Thus, *Xenopus* oocytes provide a novel and useful approach to the study of brain receptors.

Introduction

We have shown recently that, if messenger RNA (mRNA) is extracted from the electric organ of *Torpedo*, or from cat muscle, and then injected into *Xenopus laevis* oocytes, the exogenous mRNA is translated by the oocytes' own protein-synthesizing machinery and functional acetylcholine (ACh) receptor–channel complexes appear in the oocyte membrane (Barnard *et al.* 1982; Miledi & Sumikawa 1982; Miledi *et al.* 1982). In those experiments the mRNA was derived from sources rich in nicotinic ACH receptors and, accordingly, the receptors induced were of the nicotinic type. It was important to establish whether this capacity of the oocytes was limited to the synthesis of 'peripheral' nicotinic ACh receptors or whether the oocytes could be induced to incorporate in their membrane other transmitter receptors, such as those operating at synapses in the central nervous system.

To explore this question, mRNA extracted from any part of the brain would have been suitable. However, for our first attempts we chose to extract the mRNA from the optic lobe of the chick, because this is known to contain nicotinic ACh receptors, which can be labelled with α-bungarotoxin, and which are involved in retinotectal transmission in vertebrates (Schmidt & Freeman 1980; Norman *et al.* 1982). So far we have failed to obtain clear evidence that nicotinic (ACh receptors are induced in *Xenopus* oocytes after the injection of chick optic lobe (c.o.l.) mRNA; but we have discovered that the membrane of the injected oocytes acquired receptors to γ-aminobutyric acid (GABA).

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Methods

Poly(A)-mRNA was extracted from the optic lobe of 19 day old chick embryos, by homogenization, phenol extraction and chromatography in oligo(dT)-cellulose. *Xenopus* oocytes were injected, with 25–75 ng of poly(A)-mRNA in water or Hepes buffer (5 mM, pH 7.5), and kept in Barth's medium until used for electrophysiological experiments. These were made with the oocytes in normal frog Ringer solution, buffered with Hepes (5 mM, pH 7.2), at room temperature (17–25 °C). The oocyte was impaled with two microelectrodes and studied, usually under voltage clamp. The clamp current was recorded through a 500 Hz filter and fed into a tape recorder for subsequent analysis. Drugs were applied by iontophoresis from a micropipette or by bath application with use of a 1–2 ml bath and a perfusion system (3–9 ml/min). For further details of methods see Miledi & Sumikawa (1982), Miledi (1982) and Kusano *et al.* (1982).

Results

It is known that some *Xenopus* oocytes are sensitive to neurotransmitter substances such as ACh, (−)-epinephrine and dopamine, owing to the presence of the corresponding receptors in the oocyte membrane (Kusano *et al.* 1977, 1982). Initially, no responses were observed to the amino acids GABA, glycine, glutamate and aspartate applied at concentrations up to 10^{-3} M (Kusano *et al.* 1982). However, in subsequent experiments (R. Miledi & K. Sumikawa, unpublished) responses were obtained to those, as well as other, amino acids at 10^{-2} M and sometimes lower concentrations. These results will be published at a later date; suffice to say here that *Xenopus* oocytes were not often sensitive to GABA, and when they were sensitive their responses were quite small. Moreover, as with the muscarinic ACh receptors, some donors yield oocytes devoid of GABA receptors.

Induction of GABA *sensitivity*

When GABA was applied to oocytes that had been injected, a day or two earlier, with c.o.l. mRNA, their membrane was depolarized (figure 1*a*) and an inward membrane current was generated (figure 2*a*). This occurred even when the control non-injected oocytes were insensitive to GABA. The oocytes still responded to GABA after the follicular and other enveloping cells were removed by collagenase treatment, which indicates that the GABA sensitivity resides in the oocyte membrane itself.

The induction of GABA sensitivity in the injected oocytes was not caused by activation of the oocytes' own genome, because the oocytes became sensitive to GABA even when they were exposed continuously to actinomycin D to prevent synthesis of mRNA. Nor can the induction of GABA sensitivity be ascribed to the injection *per se*, because oocytes injected with mRNA from cat muscle did not become sensitive to GABA. From all this we conclude that after injection of c.o.l. mRNA the membrane of the oocyte acquires GABA receptor–channel complexes, and that this process is caused by the injection of a specific mRNA.

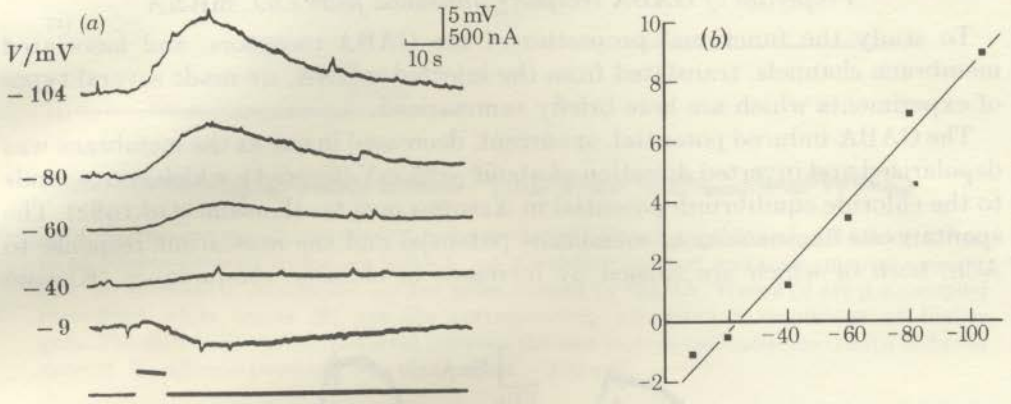

FIGURE 1. Membrane potential changes produced by iontophoretic pulses of GABA applied to an oocyte injected with c.o.l. mRNA.

(*a*) Potentials evoked by GABA when the membrane potential was set to the potentials (*V*) indicated. Lowest trace shows the iontophoretic current flowing through the GABA pipette. Vertical scale bar indicates 5 mV (GABA potentials) and 500 nA (iontophoretic current).

(*b*) Peak amplitude of GABA potentials elicited by a constant pulse of GABA applied at different membrane potentials.

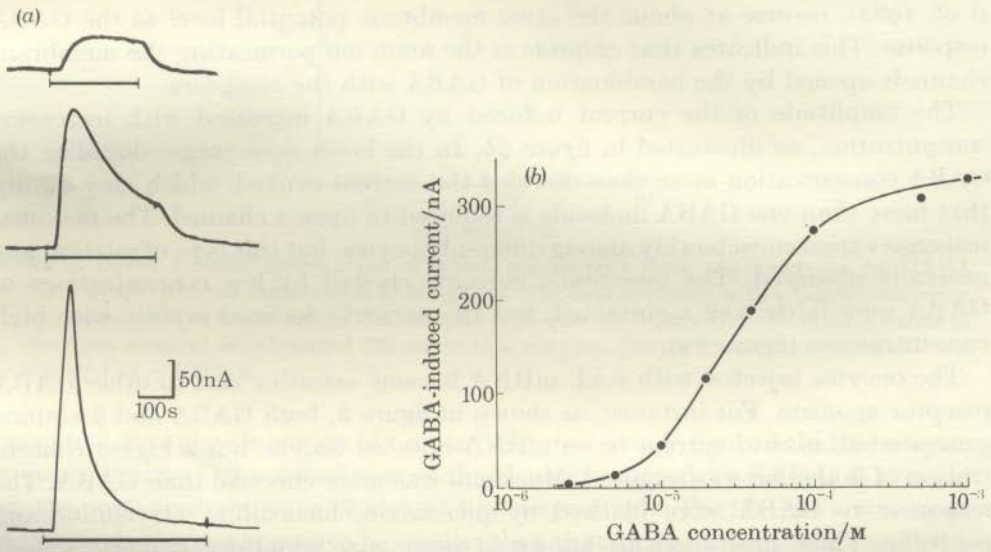

FIGURE 2. Membrane currents induced by different concentrations of GABA applied by bath perfusion to an oocyte injected with c.o.l. mRNA. (*a*) Sample GABA currents. Inward current is denoted by an upward deflexion of the trace. GABA was applied by bath perfusion for the durations indicated by the bars. The GABA concentrations were 10^{-5}, 4×10^{-4} and 10^{-3} M, from top to bottom respectively. Note that with the higher concentrations of GABA the current declined even though the drug was continuously applied. (*b*) Relation between GABA concentration and peak membrane current. Membrane potential clamped at −100 mV.

Properties of GABA *receptors translated from c.o.l.* mRNA

To study the functional properties of the GABA receptors, and associated membrane channels, translated from the injected mRNA, we made several types of experiments which are here briefly summarized.

The GABA-induced potential, or current, decreased in size as the membrane was depolarized and inverted direction at about −25 mV (figure 1), which corresponds to the chloride equilibrium potential in *Xenopus* oocytes (Kusano *et al.*1982). The spontaneous fluctuations in membrane potential and the muscarinic response to ACh, both of which are caused by increases in chloride conductance (Kusano

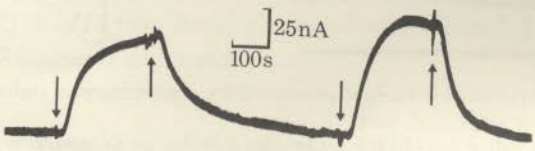

FIGURE 3. Membrane currents evoked by bath-aplied GABA and β-alanine to an oocyte injected with c.o.l. mRNA. At the downward arrows the perfusion was switched, first to β-alanine (10^{-3} M) then to GABA (10^{-5} M). At the upward arrows the perfusion was switched back to normal Ringer solution. Note that about 100 times more β-alanine was needed to evoke a current like the one induced by the GABA. Membrane potential was clamped at −100 mV.

et al. 1982), reverse at about the same membrane potential level as the GABA response. This indicates that chloride is the main ion permeating the membrane channels opened by the combination of GABA with the receptors.

The amplitude of the current induced by GABA increased with increasing concentration, as illustrated in figure 2*b*. In the lower dose range, doubling the GABA concentration more than doubled the current evoked, which may signify that more than one GABA molecule is required to open a channel. The maximal response varied considerably among different oocytes, but this type of relation was generally observed. The membrane currents elicited by low concentrations of GABA were fairly well maintained, but the currents declined rapidly with high concentrations (figure 2*a*).

The oocytes injected with c.o.l. mRNA became sensitive also to other GABA receptor agonists. For instance, as shown in figure 3, both GABA and β-alanine generated an inward current in an mRNA-injected oocyte, but a higher concentration of β-alanine was required. Muscimol was more effective than GABA. The responses to GABA were blocked by picrotoxin, bicuculline, strychnine and penicillin. These substances are known to antagonize inhibitory synaptic actions in the central nervous system of vertebrates (for reviews see: Obata 1972; Krnjević 1974; Nistri & Constanti 1979).

During the membrane current elicited by GABA, in the mRNA-injected oocytes, there was a concomitant increase in membrane current noise (figure 4). This noise is analogous to that first observed in skeletal muscle during application of ACh and was subjected to similar statistical analysis (cf. Katz & Miledi 1972; Miledi *et al.* 1982). The average power spectrum of the GABA current noise, induced in

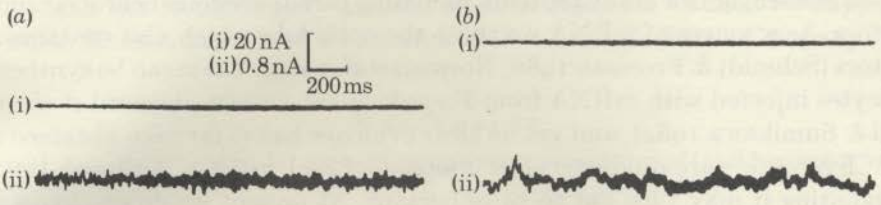

FIGURE 4. Increase in membrane current noise produced by bath application of GABA (10^{-6} M) to an oocyte injected with c.o.l. mRNA. (*a*) Membrane current and instrumental noise at rest. (*b*) Increase in membrane current noise elicited by GABA. Traces (i) are d.c.-coupled recordings while traces (ii) are the corresponding a.c.-coupled recordings at higher gain. The shift in direct current level between the two records indicates the GABA-induced current. Membrane potential was clamped at −100 mV.

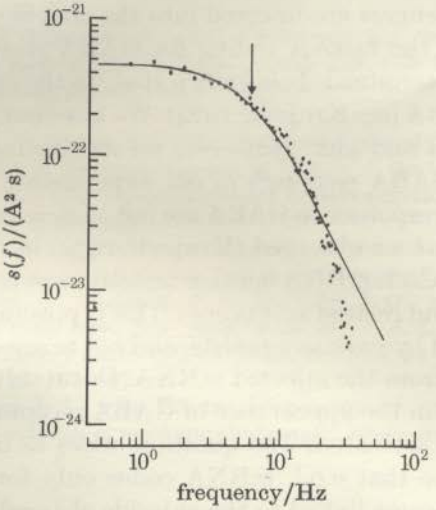

FIGURE 5. Power spectral distribution of membrane current noise ($s(f)$) induced by GABA (10^{-6} M) aplied to the same oocyte as in figure 4. The data are fitted with a Lorentzian curve by computer. The arrow marks the half-power frequency, from which the mean lifetime of the open channel is calculated (25 ms in this oocyte). Oocyte membrane potential was clamped at −100 mV.

an oocyte 3 days after injection of c.o.l. mRNA, is shown in figure 5. In this oocyte, the mean lifetime and conductance of the channels opened by GABA, as derived from the noise analysis, were 25 ms and 4 pS respectively. The lifetime is similar to but the conductance appears to be smaller than that of channels opened by GABA in mouse spinal neurons in culture (Barker & McBurney 1979).

DISCUSSION

The central finding in the present work is that *Xenopus laevis* oocytes are able to translate a mRNA coding for a transmitter receptor, which is perhaps the most ubiquitous one in the central nervous system.

The poly(A)-mRNA that was injected into the oocytes is a crude fraction which

contains messengers for many proteins, including perhaps several neurotransmitter receptors. As a source of mRNA we chose the optic lobe which also contains ACh receptors (Schmidt & Freeman 1980; Norman *et al.* 1982). These can be synthesized by oocytes injected with mRNA from *Torpedo* or cat muscle (Barnard *et al.* 1982; Miledi & Sumikawa 1982), and yet no clear evidence has so far been obtained that new ACh receptors are produced after injection of c.o.l. mRNA. Although this was disappointing it may turn out to be important. At present we do not know why just GABA, and not other receptors, were translated. It is possible that no other messengers coding for transmitter receptors were present in the mRNA preparation used; alternatively a population of messengers may have been present but was 'masked' at the time of extraction, or became subsequently masked in the oocyte by a process that selects one or more messengers for translation and incorporation of the products into the cell membrane. It will be interesting to see what happens when more specific messengers are injected into the oocyte, alone or with others.

The cellular origin of the mRNA coding for GABA receptors in the injected oocytes remains to be determined. It is known that, in the cat, both neuronal and glial cells respond to GABA (see Krnjević 1974). We have not yet extracted mRNA separately from neurons and glia. However, we are inclined to think that the mRNA coding for the GABA receptors in our experiments is of neuronal origin because in glial cells the responses to GABA are not associated with the membrane conductance changes that we observed (Krnjević 1974) in the oocyte.

Oocytes injected with c.o.l. mRNA became sensitive not only to GABA but also to β-alanine, muscimol and related substances. This is presumably due to the same receptors being activated by various agonists, and not to separate receptor entities having been translated from the injected mRNA. Our results show that injection of c.o.l. mRNA resulted in the appearance of GABA receptors that open chloride channels when they are activated. The question arises as to where the channels derive from? It could be that c.o.l. mRNA codes only for the GABA receptor protein(s) which then become linked to the chloride channels which are known to exist already in the oocyte membrane (Kusano *et al.* 1982). Alternatively the mRNA injected may include also the message for a specific channel protein, or the channel may result from the arrangement of receptor protein subunits in the membrane.

The basic properties of the chick brain GABA receptors in the oocyte resemble those of GABA receptors in the mammalian brain. For instance, both operate through an increase in membrane permeability to chloride ions: the equilibrium potential in the oocyte is much lower than in the neurons, but this probably only reflects their different intracellular concentration of chloride ions (Kusano *et al.* 1982; Krnjević 1974; Obata 1972). The molecular kinetics of GABA receptor activation are more difficult to compare because relatively little is known about the functional characteristics of GABA receptor–channel complexes in the neurons. This in itself shows the potential uses of the present approach for the study of transmitter receptors. The oocyte system makes it possible to examine the molecular processes related to synthesis, assembly and membrane incorporation of receptor–channel complexes; and once these are in such a large cell as the *Xenopus* oocyte many other functional studies are possible, which would be difficult to perform in the 'native' cells.

We thank the Royal Society and the Medical Research Council for supporting this work. K. Sumikawa was a Research Fellow of G. D. Searle Company.

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