Proc. R. Soc. Lond. B **225**, 99–106 (1985)
Printed in Great Britain

Messenger RNA from bovine retina induces kainate and glycine receptors in *Xenopus* oocytes

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(*Received* 2 *January* 1985)

The retina contains several types of nerve cells that communicate through chemical synapses. The transmitter and receptor molecules that mediate signal transmission across these synapses need further characterization. For this purpose, poly $(A)^+$ mRNA was isolated from bovine retinas and injected into *Xenopus laevis* oocytes. Translation of the foreign mRNA induced the oocyte membrane to acquire functional receptors to kainate and, to a lesser extent, also receptors to glycine, γ-aminobutryic acid (GABA), aspartate and glutamate. Thus, the cells in the retina must contain different messengers coding for these neurotransmitter receptors. Activation of the kainate receptors opens membrane channels, generating an ionic current which has an equilibrium potential close to 0 mv. The current is well maintained during prolonged application of kainate, and hence these receptors may be involved in the neurotoxic effects produced by kainate in the retina.

Introduction

The retina of the vertebrate eye contains at least six main types of nerve cells, whose morphology and interconnections are well known. When the photoreceptor cells are stimulated by light, the nerve cells communicate through arrays of chemical synapses, where signal transmission is mediated by the presynaptic release of neurotransmitters that act on receptors embedded in the postsynaptic cell membrane. Several substances, which are thought to be neurotransmitters in the brain, are probably involved in the retina (Daw *et al.* 1982; Ehinger 1982; Lasater & Dowling 1982; Osborne 1982). However, our knowledge about the receptor proteins and their mode of action is still in its infancy, due partly to the small size of the nerve cells. Recently, we succeeded in 'transplanting' transmitter receptors from nerve cells of the chick, fish, rat and human brains into the membrane of *Xenopus* oocytes, which, because they are about 1 mm in diameter, are easily amenable to a variety of experimental approaches (Gurdon *et al.* 1971; Lane 1983). This transplantation was done by isolating poly $(A)^+$ mRNA from the brains and injecting it into the oocyte. The mRNA was translated by the oocyte, which also executed postranslational modifications of the proteins and incorporated

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them into the oocyte membrane, where they formed a variety of functional receptors (Miledi *et al.* 1982*a*, 1983; Gundersen *et al.* 1983*a*, 1984*a*, *b*, *c*, *d*; Sumikawa *et al.* 1984*a*, *b*). By using this approach, we are now beginning to study the neurotransmitter receptors of retinal cells.

In this work, some confusion may arise from the ambiguity of the word receptor. Here, we use the term to refer to neurotransmitter activated receptors, and refer to the light-sensitive cells as photoreceptors.

Methods

Isolation of mRNA and injection into oocytes

Ten bovine eyes were obtained from the slaughterhouse, placed in ice and transported in the dark to the laboratory. Under red light, the eyes were transected and the retinas dissected out. After removing the pigment epithelium, poly $(A)^+$ mRNA was isolated from the retinas by using phenol–choloroform extraction and oligo (dT)-cellulose chromatography (Miledi & Sumikawa 1982). Oocytes of *Xenopus laevis* were each injected with 30 ng of mRNA, and incubated at 14–15 °C.

Electrophysiology

Three to ten days after injection the oocytes were used for electrophysiological study, as previously described (Kusano *et al.* 1982; Miledi 1982). Before examination, oocytes were usually treated with collagenase to remove follicular and other enveloping cells (Miledi & Parker 1984). Similar results to those described were also obtained from untreated oocytes. Membrane currents were recorded under voltage clamp in response to sequential application of drugs in normal frog Ringer solution. The membrane potential was usually held at -60 mV, and the temperature was 18–20 °C.

Results

Kainate receptors induced by bovine retina mRNA

Control, non-injected, oocytes of *Xenopus laevis* give only small responses, or no responses at all, when they are exposed to millimolar concentrations of amino acids and related compounds (Gundersen *et al.* 1984*a*; Kusano *et al.* 1982). In contrast, a few days after injecting the oocytes with retinal mRNA, application of kainate elicited inward membrane currents (figure 1*a*) that resembled those seen previously in oocytes injected with mRNA isolated from the brains of various animal species (Gundersen *et al.* 1984*a*, *c*; Sumikawa *et al.* 1984*b*; Houamed *et al.* 1984). A characteristic feature of the responses was that they were very repeatable. Furthermore, the membrane currents elicited by kainate were well maintained, even during exposures to high concentrations lasting many minutes. This indicates that, in contrast to other receptor-channel systems that have been induced in *Xenopus* oocytes (for example, GABA, acetylcholine and glycine (Gundersen *et al.* 1984*b*; Miledi *et al.* 1982*a*, *b*)), the kainate receptor-channel complex does not desensitize much when activated by comparatively high agonist concentrations.

Dose–response relationships were obtained by exposing the oocytes to various concentrations of kainate (figure 1*a*). At low concentrations, the amplitude of the membrane current increased more than linearly with increases in concentration (figure 2); possibly because more than one kainate receptor needs to be activated to open a membrane channel. As the kainate concentration was raised the

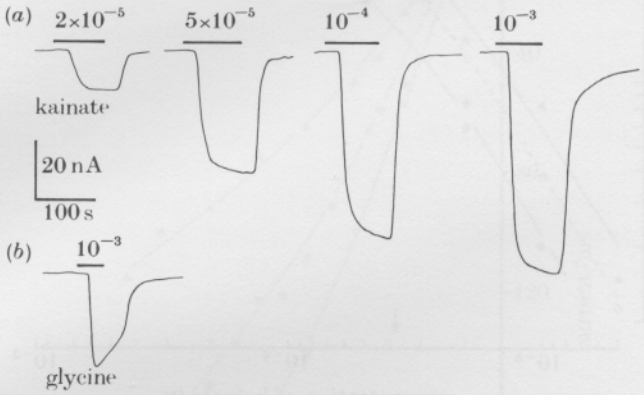

FIGURE 1. Kainate and glycine sensitivity induced in an oocyte after injection of bovine retina mRNA. Membrane currents are shown in response to kainate (*a*) and glycine (*b*), applied by bath perfusion at the molar concentrations indicated and for the times indicated by the bars. The oocyte membrane potential was clamped at −60 mV, and downward deflexions in the traces correspond to inward membrane currents.

corresponding increases in membrane current became smaller until a maximum response was reached at about 10^{-3} M. The maximum response varied greatly between different oocytes, probably due to variations in the number of kainate receptors that had been incorporated into the oocyte membrane. As shown in figure 2, about one half of the membrane channels were opened at a kainate concentration of 4×10^{-5} M, despite variations in maximum response size. This apparent dissociation constant is similar to that of kainate receptors induced by chick optic lobe mRNA (figure 2), but is smaller than for kainate receptors induced by rat and human brain mRNA (Gundersen *et al.* 1984*a*, *c*).

Effect of membrane potential on kainate current

To study the effects of membrane potential on the current evoked by kainate, the clamped potential (−60 mV) was displaced briefly to various levels before and during a steady application of kainate. The amplitude of the kainate-activated current varied linearly over a range of potentials, but deviated markedly from linearity at positive potentials (figure 3), where the response failed to increase, or even decreased, as the potential was raised. At hyperpolarized potentials (−100 mV) the current also increased less than linearly with potential, but this effect was less marked than at positive potentials. Thus, as the driving force across the membrane was increased at positive and high negative potentials, the kainate current, instead of increasing, sometimes even became smaller; as if some of the membrane channels were being closed or were rectifying. This rectification of the

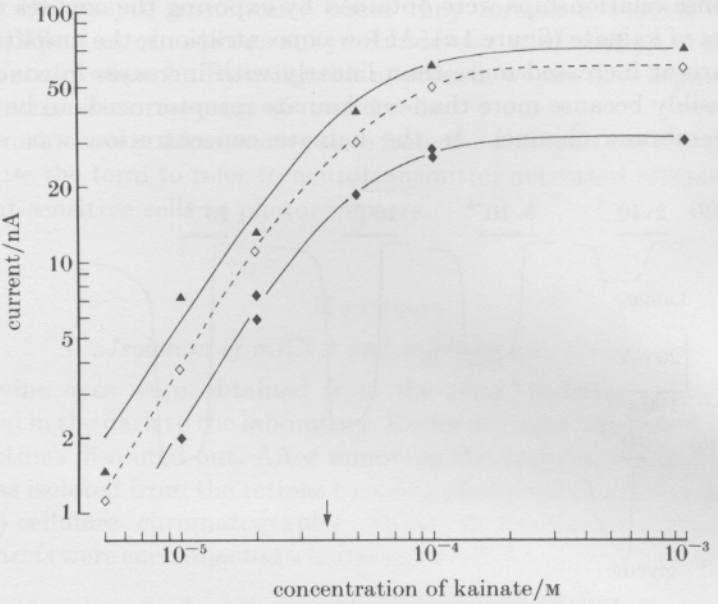

FIGURE 2. Dose–response relationship of the current elicited by kainate in two oocytes (filled symbols) injected with bovine retina mRNA. Currents were measured with the membrane clamped at −60 mV, from records as in figure 1*a*. Double logarithmic plot. The curves were drawn by eye, and at low concentrations have slopes of about 1.6. The arrow indicates the kainate concentration giving half-maximal activation in both oocytes. For comparison, data are also included from an oocyte injected with mRNA from chick optic lobe (open symbols and dashed curve). These points are shown displaced downward by one decade; that is the response at 10^{-3} M kainate was 540 nA.

overall membrane current resembles that described previously for other drug-activated channels, such as those operated by acetylcholine, serotonin, glutamate and glycine (Kusano *et al.* 1982; Gundersen *et al.* 1983*a*, 1984*b*; Miledi *et al.* 1980).

The kainate current inverted direction at about −10 mV (figure 3). A similar equilibrium potential for kainate action was obtained previously in oocytes injected with mRNA isolated from adult rat and foetal human brain (Gundersen *et al.* 1984*a*, *c*) and also from chick brain (I. Parker, K. Sumikawa and R. Miledi, unpublished data). This suggests that in all cases the membrane channels opened by kainate have similar ionic selectivity, and are permeable mainly to sodium and potassium (Gundersen *et al.* 1984*a*). In the present experiments, we found that manganese (5 mM) caused a small decrease in the amplitude of the kainate current. This would be expected if the kainate current is carried partly also by calcium ions. However, oocytes bathed in an isotonic solution where sodium was completely substituted by barium gave kainate-induced currents less than 5% of that in normal Ringer (at −60 mV). The effect of manganese is probably due, therefore, to a decrease in sodium current through the kainate-activated channels, rather than to a block of calcium influx.

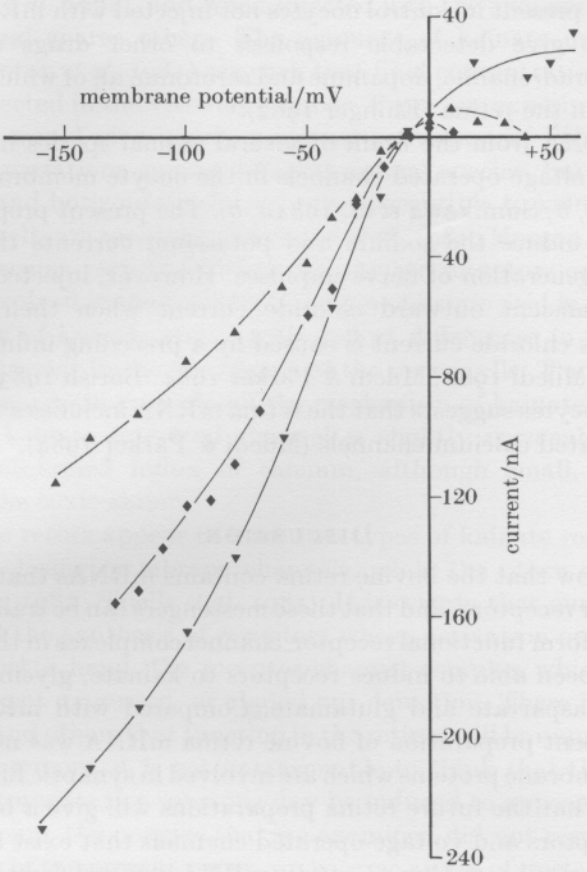

FIGURE 3. Current–voltage relationship of the current induced by kainate (10^{-4} M) in three mRNA injected oocytes (different symbols) from two donors. Measurements were made by holding the oocytes at −60 mV and applying pulses (5 s duration) to various potentials before and during drug application. The points indicate the kainate-activated current at each potential, after subtraction of 'leakage' currents.

Glycine and other transmitter receptors

In addition to inducing kainate receptors, the bovine retina mRNA induced these oocytes to acquire also glycine receptors. When glycine was applied to oocytes previously injected with retina mRNA, it elicited a current that desensitized during sustained glycine application (figure 1*b*). This current was similar to that elicited by glycine in oocytes injected with human or rat brain mRNA, and which is carried mainly by chloride ions (Gundersen *et al.* 1984*b*; Sumikawa *et al.* 1984*b*).

Our present sample of bovine retina mRNA induced mainly receptors to kainate and glycine. However, some oocytes gave small responses to GABA, L-aspartate and L-glutamate, suggesting that the retina contains also active messengers coding for these receptors. Quisqualate gave small inward currents at micromolar concentrations, and at millimolar concentrations the response became biphasic, showing an additional later outward current. The outward current, but not the

inward, was also present in control oocytes not injected with mRNA. The injected oocytes did not give detectable responses to other drugs tested, including acetylcholine, noradrenaline, dopamine and serotonin, all of which presumably act as transmitters in the retina (Ehinger 1982).

Poly $(A)^+$ mRNA from the brain of several animal species has been found to induce various voltage-operated channels in the oocyte membrane (Gundersen *et al.* 1983*b*, 1984*a*, *b*; Sumikawa *et al.* 1984*a*, *b*). The present preparation of retina mRNA did not induce the sodium and potassium currents that are normally involved in the generation of nerve impulses. However, injected oocytes showed an increased transient outward chloride current when their membrane was depolarized. This chloride current is caused by a preceding influx of calcium ions into the oocyte (Miledi 1982; Miledi & Parker 1984; Barish 1983, and its increase in the injected oocytes suggests that the retina mRNA includes a messenger coding for voltage-operated calcium channels (Miledi & Parker 1984).

Discussion

Our results show that the bovine retina contains mRNAs that code for various neurotransmitter receptors; and that these messengers can be translated effectively in the oocyte, to form functional receptor-channel complexes in the cell membrane. So far we have been able to induce receptors to kainate, glycine and sometimes also to GABA, aspartate and glutamate. Compared with mRNAs from other sources, our present preparation of bovine retina mRNA was not very potent in inducing the membrane proteins which are involved in synaptic function. However, it is very likely that the future retina preparations will give a better yield of the transmitter receptors and voltage-operated channels that exist in retinal cells. It will be particularly interesting to study mRNA derived from different species whose retinas contain mainly rod or cone photoreceptors.

The main receptor induced by bovine retina mRNA was that to kainate; which was also a preponderant receptor type induced by mRNA from fish, chick, rat and human brains (Gundersen *et al.* 1984*a*, *b*, *c*, *d*; Sumikawa *et al.* 1984*b*; Gundersen *et al.* 1983*a*, *b*). All this points to the existence of a large number of kainate receptors in cells of the central nervous system, although the cell types still need to be clearly identified. Some nerve cells in the retina are sensitive to glutamate, aspartate, kainate and quisqualate (Lasater & Dowling 1982; Wu & Dowling 1978; Ishida *et al.* 1984), but it is still not clear whether these substances act on the same, or different receptor molecules. Our present experiments, and those described previously (Gundersen *et al.* 1984*a*; Sumikawa *et al.* 1984*a*) indicate that the kainate receptor has its own identity and differs from receptors to glutamate. In mRNA injected oocytes, not only are the membrane currents induced by glutamate and kainate different in time course and ionic basis (Gundersen *et al.* 1984*a*; Sumikawa *et al.* 1984*a*), but some oocytes responded to kainate, and not to glutamate, aspartate or quisqualate. This indicates that these receptors are coded by different mRNAs, which can be purified and used for cloning the corresponding genes.

Kainic acid is a potent neurotoxic agent, which is strikingly selective for some

nerve cells (Olney *et al.* 1974), and when injected into the eye, kainate kills some nerve cell types and spares others. The amounts of kainate injected in such experiments (Hampton *et al.* 1981) are such that final concentrations of 10^{-4} M or more would be expected in the vitreous humour. From our experiments (figure 2), this concentration would give nearly maximal activation of kainate receptors. There are marked variations among different animal species, but in general the amacrine, bipolar and horizontal cells are very susceptible to kainate, while rod, cone and ganglion cells are resistant (Hampton *et al.* 1981; Morgan 1983; Schwarz & Coyle 1977). It seems very likely that the kainate receptors expressed in the oocyte are those involved in the neurotoxic action of kainic acid in the retina, and that the selectivity of kainate may simply reflect differences in the number of kainate receptors present in the membrane of the nerve cells. The oocyte system will be useful to examine in more detail the mechanism of kainate neurotoxicity. We find that the kainate-activated channel is slightly permeable to divalent cations, and a maintained influx of calcium, although small, may play an important role in the toxic action.

Nerve cells in the retina appear to have two types of kainate receptors: one of these operates by closing membrane channels, while the other opens channels (Lasater & Dowling 1982; Shiells *et al.* 1981). It is certain that our retina mRNA preparation caused the synthesis of receptors whose activation opens membrane channels. On the other hand, the receptor-channel complex which is closed by kainate was either not expressed, or eluded our detection. There is a fair chance that this receptor, and others that function in the retina, will be expressed in future experiments. Furthermore, it is not unreasonable to think that the mechanisms involved in photo transduction may one day be induced to appear in the oocyte; but, for the time being, the oocytes that we examined did not respond to light.

After completion of the present paper, we became aware of two abstracts (Noell & Labarca 1984*a*, *b*) which describe the successful induction of neurotransmitter receptors in the oocyte following injection of mRNA from chick retina.

We are grateful to Dr R. A. Shiells for useful discussion and for help in obtaining the retinas. We thank the Wellcome Trust and the Royal Society for support.

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