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Glutamate and kainate receptors induced by rat brain messenger RNA in *Xenopus* oocytes

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Xenopus laevis oocytes injected with poly(A)$^+$ mRNA extracted from rat brain became sensitive to serotonin, glutamate, kainate, acetylcholine and γ-aminobutyrate. Application of these substances to mRNA-injected oocytes elicited membrane currents. The glutamate- and acetylcholine-induced currents usually showed oscillations, while the kainate current was smooth. The current oscillations during glutamate application reversed direction at about the chloride equilibrium potential (−24 mV), but the reversal potential for the kainate current was close to 0 mV. The current–voltage relation for the glutamate-induced current oscillations showed strong rectification at hyperpolarized potentials, while that for the kainate current was nearly linear. In some oocytes, glutamate elicited smooth membrane currents, with oscillations either absent, or appearing after a delay. The reversal potential of this component was close to 0 mV, and was clearly different from that of the oscillatory component.

The appearance of glutamate and kainate sensitivity in the oocyte membrane is due to the translation of the foreign messenger RNA, and not to activation of the oocytes' own genome, because oocytes still become sensitive when transcription is prevented by enucleation or by treatment with actinomycin D. It appears that mRNA from rat brain contains translationally active messengers which code for various neurotransmitter receptors. When this mRNA is injected into *Xenopus* oocytes, the messengers are translated and receptors are inserted into the oocyte membrane, where they form functionally active receptor-channel complexes.

Introduction

We have recently shown that *Xenopus laevis* oocytes injected with poly(A)$^+$-messenger RNA (mRNA), extracted from rat brain, became sensitive to 5-hydroxytryptamine (5-HT, serotonin) (Gundersen *et al.* 1983*a*). In some of those experiments we exposed the oocytes to other putative neurotransmitters, such as noradrenaline, γ-aminobutyric acid (GABA), dopamine, acetylcholine (ACh), and glutamate, but failed to see any clear responses, beyond what is occasionally seen with these substances in 'native' oocytes. In subsequent experiments we noted that, in addition to serotonin receptors, the rat brain mRNA induced the oocyte membrane to acquire other transmitter receptors.

Methods

Procedures for extraction and enrichment of poly(A)$^+$-mRNA from brains of Wistar rats, and its injection into oocytes of *Xenopus laevis*, were as described previously (Miledi & Sumikawa 1982; Gundersen *et al.* 1983*a*,*b*). Oocytes were voltage clamped using a two-electrode system, and were continuously perfused with Ringer solution at temperatures of 15–27 °C while recording. Drugs were applied in the perfusing solution. Unless otherwise noted, all experiments using glutamate were made with the (L)-isomer. Drugs were obtained from the Sigma Chemical Company, except for APB from Calbiochem, and methysergide which was a gift from Sandoz. For further details of methods see Kusano *et al.* (1982), Miledi & Sumikawa (1982) and Miledi (1982). The oocyte membrane was usually clamped at a potential of −60 mV, so as to be away from the equilibrium potentials for sodium, potassium and chloride (Kusano *et al.* 1982), and thus facilitate detection of responses owing to changes in conductance of any of these ions.

Some oocytes were enucleated, following essentially the procedure of Ford & Gurdon (1977) using sterile solutions and treatment with collagenase (Sigma type 1, 2 mg ml^{-1} for 1 h at 20–24 °C) to remove follicular cells. These oocytes were injected with mRNA once it had been established that the germinal vesicle had been completely extruded. The oocytes were cultured in sterile Barth's solution (composition in mM: NaCl, 88; KCl, 1; $NaHCO_3$, 2.4; $MgSO_4$, 0.82; $Ca(NO_3)_2$, 0.33; $CaCl_2$, 0.41; Tris-HCl, 7.5, at pH 7.5) with nystatin (50 U ml^{-1}) and gentamycin (0.1 mg ml^{-1}) at 14–16 °C.

Results

Induction of several different drug receptors following injection of mRNA

Injection of exogenous mRNA into *Xenopus* oocytes leads to the synthesis and incorporation into the membrane of functional drug receptor-channel complexes. We had previously found that any particular mRNA preparation gave rise to mainly one type of drug receptor. For example, cat muscle mRNA induced nicotinic acetylcholine (ACh) receptors (Miledi & Sumikawa 1982); chick brain mRNA induced GABA receptors (Miledi *et al.* 1982*a*), and rat brain mRNA induced serotonin receptors (Gundersen *et al.* 1983*a*). However, on more detailed examination, injection of mRNA derived from rat brain was found to induce the synthesis, and incorporation into the oocyte membrane, of several drug receptors.

This is shown in figure 1*a*–*c*, which illustrates the membrane current responses induced by application of glutamate, serotonin and ACh to an oocyte which had been injected with rat brain mRNA several days earlier. In addition to these responses, some injected oocytes also showed sensitivity to kainate (see later) and to GABA. All of these responses resulted from the injection of rat brain mRNA, since injected oocytes gave responses, while control oocytes from the same donors, but not injected, or injected with mRNA from *Torpedo* electroplaques were not sensitive to these drugs – although, of course, the oocytes injected with *Torpedo* mRNA developed nicotinic receptors (Barnard *et al.* 1982).

'Native' muscarinic ACh receptors are often present in oocytes (Kusano *et al.* 1982), but non-injected oocytes from the donors used in the present experiments showed little or no response to ACh. The responses to ACh induced by the injection of rat brain mRNA were due to activation of receptors of the muscarinic type, which had properties similar to those of the native receptors. The membrane currents activated by ACh could be blocked by atropine, and showed slow oscillations which inverted direction at or close to the chloride equilibrium potential.

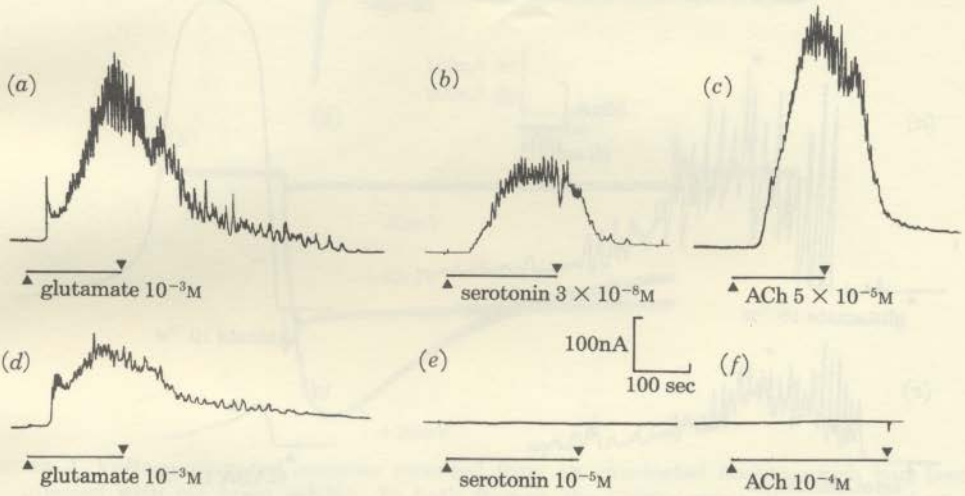

FIGURE 1. Sensitivity to glutamate (*a*), serotonin (*b*), and ACh (*c*), induced by exogenous mRNA in an oocyte which had previously been injected with rat brain mRNA. Application of drugs was for the times indicated by the bars. All traces were obtained with the oocyte voltage clamped at a potential of −60 mV. Upward deflexions in this, and other, figures denote inward membrane currents. (*a–c*) Responses obtained by application of drugs in normal Ringer solution. (*d–f*) Records obtained later from the same oocyte in the continued presence of atropine (5×10^{-7} M) and methysergide (10^{-6} M). The oocyte was perfused with atropine and methysergide for about 3 min before obtaining records, and all subsequent perfusion solutions included these drugs. The concentrations of serotonin and ACh tested in the presence of atropine and methysergide were higher than in the control. Temperature, 24 °C.

Induction of drug sensitivities does not involve the oocyte's own genome

The oocytes injected with rat brain mRNA still responded to glutamate, kainate, GABA, serotonin and ACh after the follicular and other enveloping cells were removed by collagenase treatment. This indicates that the sensitivity to these drugs resides in the oocyte membrane itself. At the same time, these experiments show that the induced receptors and channels are not destroyed by the collagenase and protease activities present in the preparation of collagenase used to defolliculate the oocytes. This contrasts with the sensitivity of native oocytes to catecholamines, which is frequently lost after collagenase treatment (Kusano *et al.* 1982).

Two main possibilities need to be considered to explain the induction of drug receptors by the injected mRNA. One is that the relevant proteins result directly from translation of the foreign mRNA by the oocyte. The other is that the injection

of foreign mRNA causes the oocyte to transcribe the appropriate messengers from its own genome. Two types of experiments indicate that the oocyte's own genome is not directly involved.

Firstly, oocytes became sensitive to all these drugs even when they were exposed continuously, for one day before and several days after the mRNA injection, to actinomycin D (50 μg ml^{-1}), to inhibit synthesis of mRNA (Adamson & Woodland 1977).

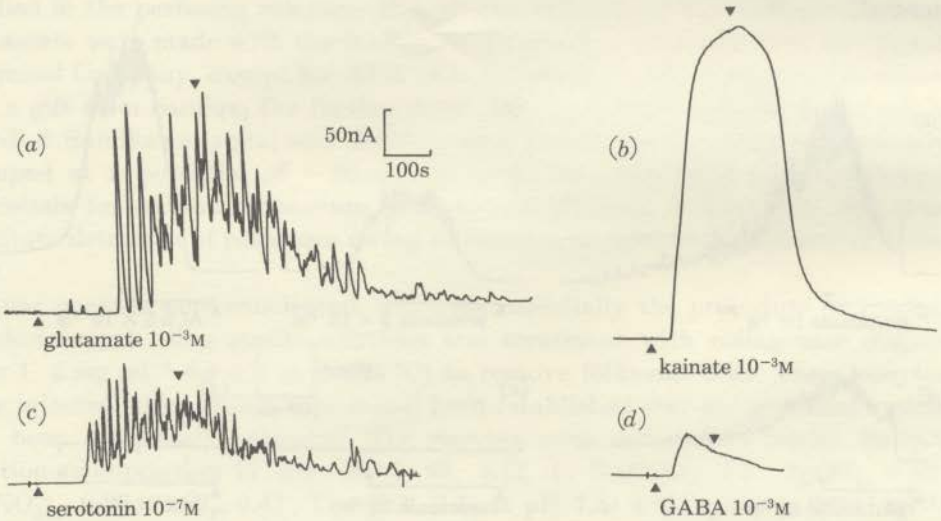

FIGURE 2. Membrane currents elicited by various drugs in oocytes which had been enucleated and subsequently injected with rat brain mRNA. Drugs were applied by bath perfusion at the concentrations indicated, beginning and ending at times shown by the arrows. Records on the left from one oocyte, and records on the right from a different oocyte. Temperature, 16 °C.

Secondly, sensitivity to drugs still developed in oocytes which had been enucleated before injection of mRNA. For these experiments, oocytes were examined two or more days after enucleation, to allow for receptors to be produced and incorporated into the membrane. The resting potential of some oocytes was normal even one to two weeks after enucleation; but in many oocytes the resting potential was only about −20 mV and their input resistance was very low. However, after inserting the microelectrodes, their resting potential and input resistance frequently increased and attained high values. A similar change is common in normal oocytes, many of which have low resting potentials after they have been kept in Barth's fluid for a few days. Presumably the increase in resting potential is triggered by an influx of calcium ions during impalement, and the process is not prevented by enucleation.

Figure 2 shows records from two enucleated oocytes which gave membrane currents in response to glutamate, kainate, GABA and serotonin. The voltage-activated sodium and potassium channels which are induced by rat brain mRNA (Gundersen *et al.* 1983*b*) were also still observed in enucleated oocytes (figure 3), as was also the transient outward current (figure 3*b*) seen in normal oocytes (Miledi 1982).

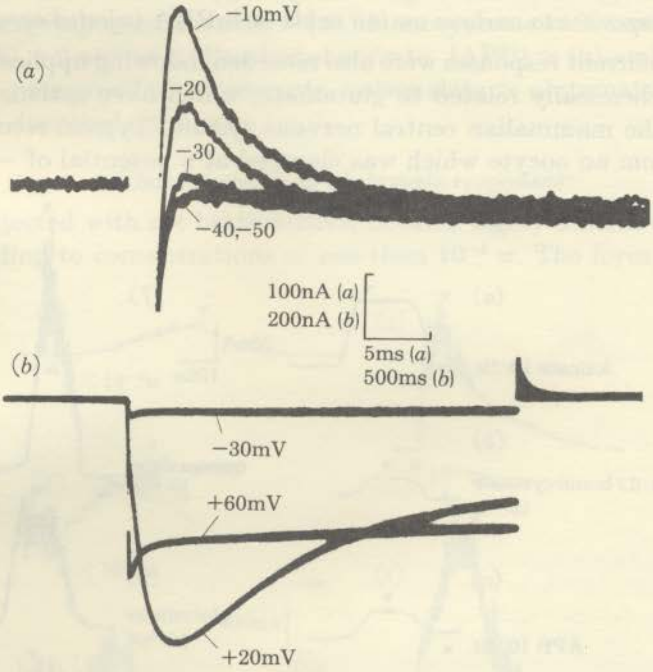

FIGURE 3. Voltage-activated currents recorded from an enucleated oocyte which had been injected with rat brain mRNA. In both frames, the oocyte was voltage clamped at a potential of −100 mV, and then stepped to the various potentials indicated. Temperature, 16 °C. Same oocyte as figure 2*b*,*d*. (*a*) Voltage-activated sodium currents (cf. Gundersen *et al.* 1983*b*). Superimposed records are shown obtained at potentials of −50, −40, −30, −20 and −10 mV. (*b*) Transient outward and potassium currents from the same oocyte, shown on a slower sweep speed. The oocyte was depolarized with a 3 s pulse to the potentials indicated. At +20 mV a large transient outward current can be seen, but this was suppressed at +60 mV, allowing the faster outward potassium current to be seen.

The glutamate response involves specific receptors

Bath perfusion of glutamate elicited slow inward membrane currents, which usually showed pronounced oscillations (figures 1*a*, 4*g*, 5, 6). These responses are quite unlike the smooth currents elicited by activation of GABA or nicotinic ACh receptors incorporated following the corresponding mRNA injections (Miledi & Sumikawa 1982; Miledi *et al.* 1982*a*,*b*), but closely resemble the oscillatory currents produced by activation of serotonin or muscarinic ACh receptors (figure 1; see also Gundersen *et al.* 1983*a*). The response to glutamate, however, does not involve these receptors, since glutamate sensitivity remained after the oocytes were treated with both atropine (5×10^{-7} M), to block ACh receptors, and methysergide (10^{-6} M) to block the serotonin receptors (figure 1*d*–*f*). The response to glutamate after adding atropine and methysergide was smaller than the original control response (figure 1*a*, *d*), but a similar variability was often seen during repeated glutamate applications in normal Ringer.

Responses to various amino acids in mRNA *injected oocytes*

Membrane current responses were also recorded following application of various compounds, chemically related to glutamate, which have actions on glutamate receptors in the mammalian central nervous system. Typical records are shown in figure 4, from an oocyte which was clamped at a potential of −60 mV. These

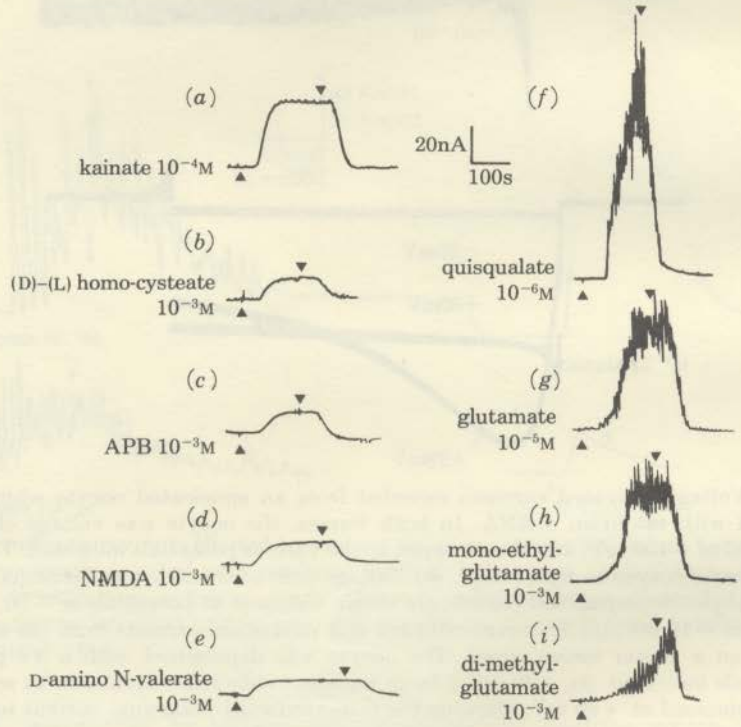

FIGURE 4. Membrane currents elicited by various amino acids. All records were obtained from the same oocyte, which was voltage clamped at −60 mV. The amino acids were applied by bath perfusion, beginning and ending as indicated by the arrows. Responses showing oscillations in membrane current are grouped on the right, while smooth current responses are shown on the left. Responses within each column are arranged in approximate order of drug potency. Temperature, 18 °C. The oocyte had been injected with rat brain mRNA, and was pre-treated with collagenase.

responses fall into two distinct categories. Some agents, such as glutamate, elicited membrane currents showing marked oscillations (figure 4*f–i*), while others, such as kainate, gave smooth currents without any clear oscillations (figure 4*a–e*). (D)-glutamate was much less potent than (L)-glutamate, and at a concentration of 10 mM usually gave no detectable response, or at most an inward current of a few nanoamperes. (D)- or (L)-aspartate, at a concentration of 10 mM, usually gave either no detectable responses, or only a small response, like that sometimes seen in control oocytes (R. Miledi & K. Sumikawa, unpublished observations). In a few oocytes (L)-aspartate gave a smooth inward current, which was presumably a consequence of mRNA injection, since control oocytes from the respective donors did not respond to aspartate.

The approximate order of potency of drugs tested was: (i) for drugs giving smooth current responses, kainate > (DL)-homocysteate ≈ *N*-methyl-(DL)-aspartate (NMDA) ≈ 2-amino-4-phosphonobutyrate (APB) ≈ (D)-amino-*N*-valerate; (ii) for drugs giving oscillatory currents, quisqualate > glutamate > monoethylglutamate ≈ dimethylglutamate.

Characteristics of glutamate responses

Oocytes injected with rat brain mRNA became highly sensitive to glutamate, some responding to concentrations of less than 10^{-6} M. The form and amplitude

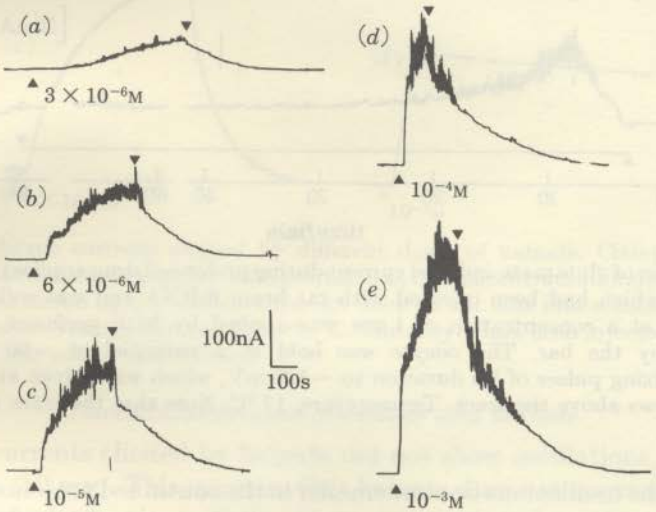

FIGURE 5. Membrane currents elicited by different concentrations of glutamate. The oocyte had been injected with rat brain mRNA, and was clamped at −60 mV. Application of drugs by bath perfusion began and ended as indicated by the arrows, although these do not take into account the dead time of the perfusion system (roughly 10–20 s). Different concentrations were tested after thoroughly washing out glutamate from each previous trial. Temperature, 26 °C.

of the membrane currents induced by glutamate showed considerable variation among different oocytes. Large oscillations, with various degrees of synchronization, were usually present, although in some oocytes oscillations were small or entirely absent. In the latter oocytes the responses to ACh and serotonin, which usually have oscillations, were often smooth; but smooth responses to glutamate were also observed in oocytes which responded with oscillations to ACh or serotonin. The time course of the responses also showed large variation. Sometimes, soon after glutamate was applied, there was an initial sharp peak (figure 1*a*), which could have an amplitude of several hundred nanoamperes; while in other cases this peak was absent, and oscillations developed slowly (figure 4*g*). Occasionally, the initial phase of the response showed a smooth inward current and oscillations began only after a delay (figures 6, 10*a*). This type of response was seen most prominently with high concentrations of glutamate (1–10 mM).

As is the case for the muscarinic responses to ACh (Kusano *et al.* 1982) and the responses to serotonin (Gundersen *et al.* 1983*a*), the maximum current amplitude during glutamate application increased steeply over a narrow concentration range.

For example, the oocyte represented in figure 5 gave no response to 10^{-6} M glutamate, but at 10^{-5} M it gave a large response, which increased only about twofold when the concentration was further raised by a factor of 100, to 10^{-3} M. The latency to onset of the oscillatory response was also concentration dependent, and at low concentrations latencies as long as 2 min were observed (figure 5*a*).

The responses to glutamate were maintained for many seconds, but during prolonged perfusion with 1 mM glutamate, the currents declined over a time course of several minutes (figure 6). Both the overall current, and the peak to peak

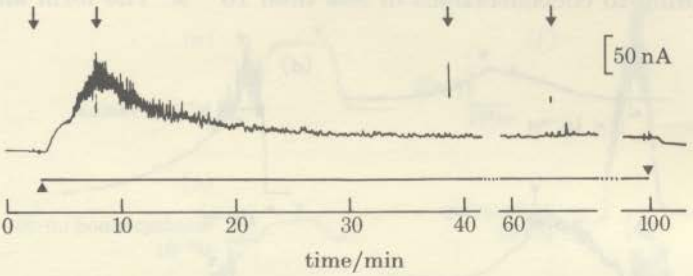

FIGURE 6. Decline of glutamate-induced current during prolonged drug application. Record from an oocyte which had been injected with rat brain mRNA and was collagenase-treated. Glutamate at a concentration of 1 mM was applied by bath perfusion for 100 min, as indicated by the bar. The oocyte was held at a potential of −60 mV, except for hyperpolarizing pulses of 3 s duration to −120 mV, which were given at times indicated by the arrows above the trace. Temperature, 17 °C. Note that the trace is interrupted at two points.

amplitude of the oscillations became smaller in the continued presence of glutamate. After about 100 min the oscillations had virtually stopped, although a small steady current remained, which declined when the glutamate was washed out. Application of serotonin (10^{-7} M) during perfusions with glutamate still elicited a large oscillatory response, at a time when the glutamate-induced oscillations had almost ceased. This shows that the capacity of the oocyte to generate oscillations had not been exhausted.

Kainate responses

Application of kainate to oocytes injected with rat brain mRNA elicited inward membrane currents (figures 4*a* and 7). These responses had a slow time course and were well maintained, even during kainate applications lasting many minutes (figures 7*f* and 8). In contrast to the currents elicited by glutamate, the kainate currents were smooth, and we did not detect clear oscillations during exposure to kainate, except for the spontaneous oscillations which were sometimes already present before drug application (cf. Kusano *et al.* 1982).

Responses elicited by different doses of bath-applied kainate are illustrated in figure 7. The minimal concentration required to elicit detectable responses was generally higher than for glutamate. For example, although the oocyte illustrated in figure 7 was particularly sensitive, kainate at 10^{-5} M failed to elicit a response, while glutamate gave responses at concentrations down to 10^{-6} M in a few oocytes. At low doses of kainate, a doubling in the concentration gave a more than twofold increase in the peak size of the response (figure 7*a–c*).

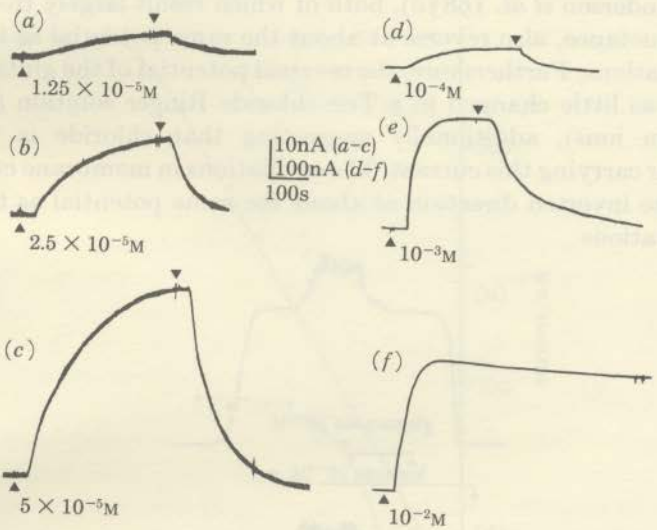

FIGURE 7. Membrane currents elicited by different doses of kainate. Clamp potential was −60 mV. Kainate was applied by bath perfusion at the concentrations indicated, beginning and ending as shown by the arrows (although these do not take into account the dead time of the perfusion system). Temperature, 18 °C. The oocyte had been injected with rat brain mRNA and was collagenase-treated.

Interactions between glutamate and kainate

Membrane currents elicited by kainate did not show oscillations, even at high concentrations (10 mM). This suggests that kainate does not appreciably activate the receptors which give rise to the oscillatory currents induced by glutamate. To ascertain that the oocyte was still able to generate any oscillations, while being exposed to a high concentration of kainate, we applied glutamate during perfusion of the oocyte with kainate. Figure 8*a* shows a record obtained by applying a low concentration of glutamate (10^{-5} M) during perfusion with kainate (10^{-3} M). Before addition of glutamate, the kainate-induced current was smooth. However, when glutamate was added it induced an oscillatory response, which summated with the steady kainate current, and appeared similar to the response elicited by glutamate alone (figure 8*b*).

In contrast to this potentiation, higher concentrations of glutamate were found to depress the membrane current induced by kainate. Figure 8*c* shows a record where 10^{-3} M glutamate was applied during perfusion with 10^{-3} M kainate. In this case, glutamate caused a fall in the membrane current, but nevertheless oscillatory currents developed.

Equilibrium potential of glutamate and kainate currents

The current oscillations induced by glutamate decreased in size as the membrane was depolarized, and inverted direction at a potential of −24.2 mV (±2.7 mV s.e. of mean, five oocytes). This corresponds to the chloride equilibrium potential in *Xenopus* oocytes (Kusano *et al.* 1982). The oscillatory responses produced by activation of muscarinic ACh receptors (Kusano *et al.* 1982) and serotonin

receptors (Gundersen *et al.* 1983*a*), both of which result largely from increases in chloride conductance, also reverse at about the same potential as the glutamate-induced oscillations. Furthermore, the reversal potential of the glutamate-induced oscillations was little changed in a Tris-chloride Ringer solution (containing no added sodium ions), additionally suggesting that chloride is the main ion responsible for carrying this current. The oscillations in membrane current induced by quisqualate inverted direction at about the same potential as the glutamate-induced oscillations.

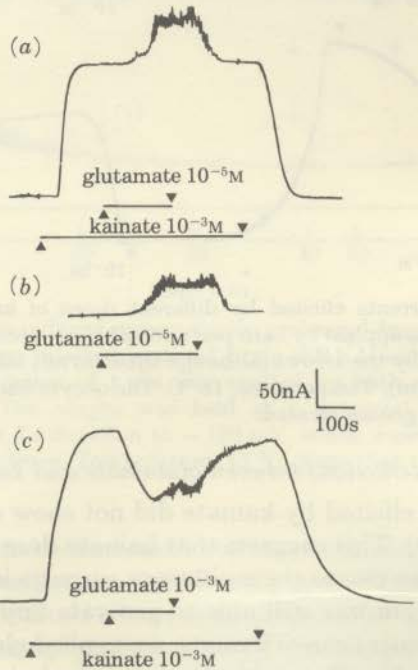

FIGURE 8. Interactions between glutamate and kainate. (*a*) A low concentration (10^{-5} M) of glutamate was applied during a longer, continuous application of kainate (10^{-3} M). Bars indicate the durations of each application. The response elicited by kainate was smooth, even though this oocyte showed a few small spontaneous oscillations. (*b*) Response to glutamate applied alone to the same oocyte as in (*a*). (*c*) A similar record to (*a*), except that a glutamate concentration of 10^{-3} M was used. Different oocyte to (*a*) and (*b*). Both oocytes had been injected with rat brain mRNA and were collagenase treated. Temperature, 18–19 °C.

The reversal potential of the overall glutamate-induced current was examined by clamping the membrane to different potentials during maintained glutamate application and noting the potential at which the drug-induced current was reduced to zero (figure 9). Oocytes with oscillatory responses to glutamate showed reversal potentials of about -20 mV measured in this way. However, other oocytes that showed smooth glutamate responses gave reversal potentials close to 0 mV. A mean reversal potential of -7.7 ± 2.0 mV was obtained from nine oocytes showing smooth responses.

The reversal potential of the kainate-induced current was measured in two ways; by stepping the membrane potential to different voltages during a long application

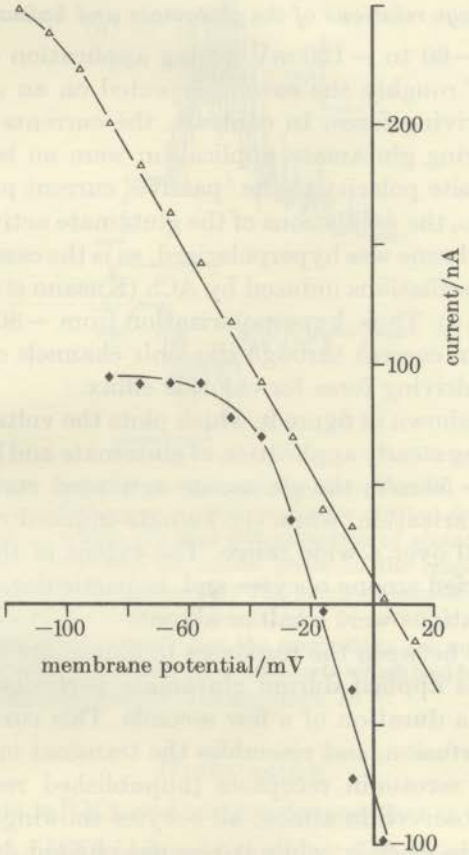

FIGURE 9. Current–voltage relations measured during steady responses to 3×10^{-4} M glutamate (filled symbols) and 2×10^{-4} M kainate (open symbols). Both measurements from the same, rat brain mRNA-injected, oocyte. Measurements were made by recording the currents in response to voltage clamp pulses to various potentials from a holding potential of −60 mV. Records were obtained before drug application and then at a time during drug perfusion when the response had risen to a stable level. The points show the drug-induced currents after subtraction of the passive currents recorded at the same potentials before drug application. The oocyte showed marked oscillations during glutamate application, and currents were measured as the estimated mean value between oscillations. Curves were drawn by eye. Temperature, 26 °C.

of kainate (figure 9), or by applying brief (1–5 s) 'puffs' of kainate through the perfusion system while holding the membrane potential at different levels. Both of these methods gave similar values, with a mean estimate of −3.2 mV (±5.0 mV, five oocytes). The reversal potential for the kainate-induced current was clearly different from that of the oscillatory glutamate response, even when both measurements were made in the same oocyte (figure 9). Reduction of the sodium concentration in the Ringer solution to one half (by equimolar substitution with Tris-chloride) changed the reversal potential to about −20 mV. In contrast, reduction of the chloride concentration to one half of the normal (substituting with acetate), gave no appreciable change in reversal potential for kainate.

Current–voltage relations of the glutamate and kainate responses

Polarization from −60 to −120 mV during application of kainate caused an increase in current of roughly the extent expected on an ohmic basis from the increased electrical driving force. In contrast, the currents elicited by the same hyperpolarization during glutamate application were no larger, and sometimes were even of the opposite polarity to the 'passive' current produced before glutamate was applied. Also, the oscillations of the glutamate activated current became smaller when the membrane was hyperpolarized, as is the case also for spontaneous oscillations, and the oscillations induced by ACh (Kusano *et al.* 1982) or serotonin (Gundersen *et al.* 1983*a*). Thus, hyperpolarization from −60 to −120 mV caused virtually no increase in current through the ionic channels opened by glutamate, despite the increased driving force for chloride efflux.

This rectification is shown in figure 9, which plots the voltage dependence of the currents induced during steady application of glutamate and kainate. At potentials more negative than −50 mV, the glutamate-activated current did not increase further with hyperpolarization, while the kainate-induced current varied almost linearly with potential over a wide range. The extent of the rectification of the glutamate current varied among oocytes and, in particular, it was less marked in cases where the oscillations were small or absent.

A further difference between the responses to glutamate and kainate, was that hyperpolarizing pulses applied during glutamate perfusion elicited a transient inward current, with a duration of a few seconds. This current increased slowly in size during drug perfusion, and resembles the transient inward current present during activation of serotonin receptors (unpublished results). The transient inward current was observed in almost all oocytes showing oscillatory responses to glutamate, serotonin or ACh, while it was not elicited during even prolonged application of kainate. During prolonged application of glutamate, the size of the transient inward current declined (figure 6). In the record shown, a pulse to −120 mV given about 40 min after beginning perfusion elicited a transient inward current of 50 nA, while the same pulse given after some 60 min elicited a current of only about 5 nA.

The glutamate response is comprised of two components

Several observations described above suggest that glutamate may activate two different types of membrane current: (i) an oscillatory current, which inverts direction at about −24 mV, and (ii) a smooth membrane current, which inverts close to 0 mV. Figure 10 shows records from an oocyte where the oscillatory response began after a long latency, and clearly demonstrates the existence of two separate components.

When clamped at a potential of −60 mV, perfusion with 10 mM glutamate elicited a smooth membrane current, followed by oscillations which commenced about 2 min later (figure 10*a*). Both the smooth and oscillatory components gave upward deflexions at this potential, corresponding to inward membrane currents. However, when the glutamate application was repeated with the oocyte clamped at −20 mV, the direction of the oscillatory component was inverted, although the initial smooth component still gave an inward current (figure 10*b*).

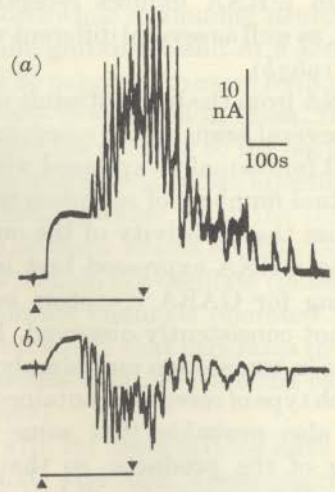

FIGURE 10. The glutamate response comprises two components, which invert direction at different potentials. (*a*) Membrane current responses elicited from an oocyte clamped at −60 mV, in response to a high (10 mM) concentration of glutamate applied for the time indicated by the bar. Note that the initial phase of the response is smooth, and that oscillations begin after a long latency. (*b*) The same glutamate application as in (*a*), but with the oocyte clamped at a holding potential of −20 mV. The initial smooth phase is still upward (inward membrane current), but the oscillations are now inverted and represent outward membrane currents. Records from an oocyte which had been injected with rat brain mRNA and was collagenase-treated. Temperature, 16 °C.

DISCUSSION

Foreign mRNA *and transmitter receptors in oocytes*

We have shown that mRNA isolated from adult rat brain induces the appearance of various transmitter receptors in the oocyte membrane. Moreover, since the receptors appeared in oocytes that had been enucleated, and also in oocytes that were exposed to actinomycin D, it appears that the receptors do not result from a triggering of transcription of mRNA from the oocytes' own genome. The possibility remains that the foreign mRNA activates a previously transcribed 'native' mRNA, or even previously synthesized, but inactive, receptor-channel proteins. However, the available evidence suggests that this is unlikely, and that the new receptors result directly from the translation of the injected mRNA.

The messenger RNA used in the present experiments, as well as that used earlier, were heterogenous preparations presumed to contain a mixture of messengers coding for various polypeptides. We were therefore surprised to find that chick brain mRNA induced essentially only GABA receptors (Miledi *et al.* 1982*a*), while rat brain mRNA induced predominantly serotonin receptors (Gundersen *et al.* 1983*a*). This could have occurred if, from a mixture of messages, the oocyte selected one for translation. To test this possibility, we had injected oocytes with mRNA from both chick brain and cat muscle. These oocytes acquired GABA as well as nicotinic ACh receptors (Miledi *et al.* 1983; and unpublished data), which indicated that mRNA for one receptor did not exclude others from being translated by the oocyte. This conclusion is greatly strengthened by the present experiments,

which show that rat brain mRNA induces receptors to serotonin, kainate, glutamate, ACh and GABA, as well as several different voltage operated membrane channels (Gundersen *et al.* 1983*b*).

It is clear then, that mRNA from the brain of adult rats contains translationally active mRNA coding for several transmitter receptors; but it is also clear that these messengers are not all functionally expressed with equal efficiency. At present we do not know the actual numbers of receptors incorporated into the oocyte membrane but, judging from the sensitivity of the oocytes to the various transmitters, it appears that the mRNA expressed best is that coding for serotonin receptors, while that coding for GABA receptors is much less efficient, since responses to GABA were not consistently observed. Kainate and glutamate lay in between. Such differences in translation may simply reflect the relative number of transcripts, coding for each type of receptor, contained in the mRNA preparations we used. However, it is also probable that some control is exerted during translation and processing of the products, so that some mRNA species are preferentially expressed. In this context it will be interesting to see if rat brain mRNA is expressed differently after injection into neurons and other cells.

Cellular source of active mRNA

Since the mRNA was isolated from whole brains, the messengers coding for the various receptors induced in the oocytes may originate in any of the brain cells: neuronal, glial, vascular, etc. However, neurons are probably the main source because, in addition to their large number, they respond to many transmitter substances. In contrast, glial cells are generally unresponsive, or their response is not associated with an increase in membrane conductance (Krnjević & Schwartz 1967; Hösli & Hösli 1978) such as that observed in the oocyte.

Activation of rat drug receptors in frog oocytes and in neurons

There is considerable information on the effects of amino acids and other substances on mammalian nerve cells (for reviews see Curtis & Johnson 1974; Krnjević 1974, 1982; Hösli & Hösli 1978; Nistri & Constanti 1979; Watkins & Evans 1981; Nelson *et al.* 1981; Macdonald & Barker 1981; Cotman *et al.* 1981), and some interesting points emerge when we compare these effects with the action of transmitters on 'rat receptors' in the oocyte. For instance, glutamate is known to excite practically all mammalian nerve cells. Nevertheless, if the glutamate receptors induced in the oocyte exist in brain neurons, these cells might be inhibited by glutamate, which opens chloride channels. It has been reported that 50 % of cerebellar interneurons in the guinea pig are excited by glutamate and 50 % are inhibited (Yamamoto *et al.* 1977). However this inhibition was not abolished in chloride-free medium, as would be expected if glutamate were acting by increasing the membrane conductance to chloride, as in the oocytes.

Much remains to be discovered about the mode of action of transmitters on mammalian neurons. It seems to us that the oocyte can contribute significantly, not only because the molecular action of transmitters can now be studied in depth, but also because results obtained in the oocyte can guide our approach to neurons.

For example, it would be worthwhile examining neurons for oscillatory currents like those elicited by serotonin, glutamate and ACh acting on the receptors in the oocyte. All these drugs might be expected to exert inhibitory actions on nerve cells, and oscillations might be easily overlooked in them. It is of course possible that the oscillatory chloride currents are peculiar to the oocytes, which already possess a mechanism able to generate oscillations before injection of mRNA (Kusano *et al.* 1982). It should be mentioned, however, that the GABA receptors induced in oocytes by mRNA from rat or chick brains act like GABA receptors in neurons and open chloride channels which do not oscillate (Miledi *et al.* 1982*a*). Thus, even in the same oocyte, the chloride channels operated by a transmitter are not necessarily coupled to the mechanism that generates the oscillations; nor, for that matter, are the chloride channels that generate the calcium-dependent outward current in oocytes (Miledi 1982).

Xenopus oocytes injected with rat brain mRNA have chloride channels that are opened by serotonin, glutamate and ACh. These channels are in addition to the chloride channels that are opened by an influx of calcium (Miledi 1982) and those that open spontaneously (Kusano *et al.* 1982). Faced with all these chloride channels it would seem economical if some of them were common to various receptors. This possibility has been considered previously for neurons by Carpenter *et al.* (1977), and by Barker & McBurney (1979). Part of the evidence adduced by the former authors was that curare blocked sodium and chloride responses of *Aplysia* neurons to various transmitters. However, the oscillatory responses of oocytes to ACh, serotonin and glutamate are not blocked by curare (Kusano *et al.* 1982; Gundersen *et al.* 1983*a*; and unpublished). The possibility of different receptors being linked to common channels remains an attractive one, and we hope that further work, including recording of single channel currents in the oocyte (Miledi *et al.* 1983) may help resolve this question.

Although ACh, serotonin and glutamate all evoked oscillatory chloride currents in mRNA-injected oocytes, the evidence available (figure 1) indicates that these substances activate different receptors. Smooth responses were obtained with kainate, GABA and rarely also with aspartate. The type of these responses, and their equilibrium potentials, indicate that these substances act on still other receptors.

It is not yet clear, however, if the smooth response to glutamate is evoked by activation of kainate receptors, or whether a separate 'excitatory' glutamate receptor was also translated. The decrease in kainate current produced by high concentrations of glutamate (figure 8*c*) suggests that glutamate may compete with kainate for the same receptor, but is less effective than kainate in opening the channels. It is also not clear whether other glutamate related substances (figure 4) act on the same receptors as kainate and glutamate, or whether different receptors are involved. Preliminary observations suggest that quisqualate may act on the same receptors, because quisqualate was generally more potent than glutamate in spite of very large differences in the degree of expression of the injected mRNA in different oocytes. Experiments in progress using antagonists may provide more definite information on these questions.

We are only at the beginning of these studies, but it already seems that inducing

the oocyte membrane to acquire transmitter receptors may help us to understand the normal and pathological functions of transmitter systems in the original nerve cells.

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