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Messenger RNA from human brain induces drug- and voltage-operated channels in *Xenopus* oocytes

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Sodium channels and receptors to serotonin and kainate were 'transplanted' from human brain into frog oocytes, by isolating messenger RNA from a fetal brain, and injecting it into Xenopus laevis *oocytes. The mRNA was translated by the oocyte and induced the appearance of functional receptors and channels in its membrane. This approach renders drug- and voltage-operated channels of the human brain more amenable to detailed study.*

FOR obvious reasons, it is important to know how neurotransmitter receptors and channels function in human brain neurones; but these cells are not easily amenable to the necessary experimental approaches. One possibility would be to culture human brain cells[1,2] and study them with electrophysiological and biochemical techniques[3–6]. Alternatively, one might purify the receptor and channel proteins from human neuronal membranes, and study their function after incorporation into artificial membranes, as has been done with other systems[7,8]. We decided on a different approach, one that may allow us to study not only the functioning of receptors and channels, but also the way in which their constituent proteins are synthesized, processed and incorporated into the cell membrane. Essentially, the method consists of isolating messenger RNA (mRNA) from the human brain, and injecting it into oocytes of *Xenopus laevis*. This induces the appearance of receptors and channels in the oocyte membrane, in the same way as was done previously with mRNA from chick[9] or rat[10–12] brains.

Membrane channels induced by mRNA

Injection of mRNA derived from fetal human cerebral cortex into *Xenopus* oocytes caused the synthesis, and incorporation into the membrane, of channels which could be activated either by drugs or by voltage changes. These included voltage operated sodium channels (Figs 1, 2), and receptor–channel complexes activated by serotonin (Fig. 3) and kainate (Fig. 4). All of them resulted from the injection of exogenous mRNA, since most injected oocytes gave responses, whilst control (non-injected) oocytes from the same donors did not show responses (Table 1).

Two further lines of evidence indicate that the proteins forming these membrane channels arose directly from the translation of the human mRNA by the oocyte, and not because the injection of foreign mRNA caused the oocyte to transcribe the appropriate messengers from its own genome. First, oocytes developed the voltage- and drug-activated responses even after they had been exposed continuously to actinomycin D (50 μg ml^{-1}) to inhibit synthesis of mRNA[13]. Second, the responses still developed in oocytes which had been enucleated[12,14] before injection of human mRNA.

Voltage-activated sodium current

A few days after injecting the oocytes with human cerebral cortex mRNA, depolarizing pulses elicited an inward membrane current (Fig. 1*a*). In contrast, non-injected oocytes from these donors showed predominantly passive (ohmic) currents. Figure 1 illustrates the current–voltage relationship for the inward current. The current first became detectable when the membrane potential was stepped from -100 to about -40 mV, and increased with increasing depolarization to reach a maximum at about -10 mV. With further depolarization the current declined, and was reduced to about zero at a potential of $+60$ mV.

The peak amplitude of the inward current elicited by depolarization was reduced if external sodium was partially substituted with Tris or tetraethylammonium ions. Furthermore, tetrodotoxin (TTX) readily abolished the inward current (complete block with 300 nM, half block with 10 nM). All this indicates that sodium is the main ion responsible for the inward current. Manganese (5 mM) also reduced the inward current, but this effect probably arises from a change in the characteristics of the sodium channels, rather than from a blockage of calcium channels[11,15].

The decay of the sodium current in oocytes injected with human cerebral cortex mRNA often showed two time courses. For example, in the upper record in Fig. 2*a*, the current shows an initial rapid decline, followed by a slower decay. During repetitive activation, the slow component of the decay 'fatigued' more readily than the fast component. This is evident in Fig. 2*a*. where two depolarizing pulses of 900 ms duration to a potential of -10 mV were separated by an interval of 100 ms. The slow component was strongly reduced during the second pulse, whilst the fast component appeared little changed.

Sodium currents induced by rat brain mRNA show a similar, two-component decay[11], but with a time course approximately three times faster. Mean values for the half decay time of sodium currents measured in oocytes from the same donor (at -10 mV and 12–15 °C) were: human cortex, 13.7 ± 1.3 ms (s.e.m., 18 oocytes; rat brain, 4.8 ± 1 ms (6 oocytes).

Table 1 Drug- and voltage-activated membrane currents induced in *Xenopus* oocytes following injection of mRNA

	Human cerebral cortex	Human cerebellum	Rat brain	Control
Sodium current	68 ± 12 (30)	6 ± 1.7 (15)	332 ± 40 (23)	0 (8)
Potassium current	0 (23)	0 (14)	331 ± 57 (23)	0 (8)
T_{out} current	22 ± 3.6 (29)	17 ± 5 (14)	341 ± 107 (24)	15 ± 3.5 (8)
Serotonin	60 ± 12 (19) [10^{-5} M]	2 (8) [10^{-5} M]	625 ± 126 (4) [10^{-7} M]	0 (6) [10^{-3} M]
Kainate	12 ± 4 (20) [10^{-3} M]	3 ± 1 (11) [10^{-3} M]	18 (4) [10^{-5} M] 250 (1) [10^{-3} M]	0 (6) [10^{-3} M]
GABA	0 (12) [10^{-3} M]	0 (7) [10^{-2} M]	277 ± 143 (4) [10^{-4} M]	0 (6) [10^{-3}]

Numbers give peak current responses in nA, with standard error of mean, and number of oocytes in parentheses. In the case of drug-activated currents, measurements were made at a clamp potential of −60 mV, and the drug concentrations are given in square brackets. The voltage-activated sodium current was measured as the peak current elicited by depolarization to a potential of −10 mV from a holding potential of −100 mV. Similarly, the potassium current and the transient outward (T_{out}) current were measured for depolarizations to, respectively, +40 and 0 mV. All oocytes, including controls (non-injected), were obtained from the same two donors. Temperature, 12–15°C.

Effects of toxins

The sodium channels induced by human mRNA in *Xenopus* oocytes were blocked by TTX and were affected by veratrine, as are the sodium channels in nerve membranes[16]. For instance, the sodium current is normally inactivated almost completely during long depolarizing pulses (Fig. 1*a*). In contrast, after veratrine some inward current remains during pulses lasting many seconds. Figure 2*b*, *c* shows currents elicited by depolarizations to 0 mV before and after adding veratrine (0.5 mg ml^{-1}) to the perfusion fluid. In the presence of veratrine a maintained inward current persisted throughout the depolarization, while the initial transient inward current was slightly smaller and showed a faster decline (Fig. 2*b*). The most striking effect was, however, seen when the oocyte was repolarized. Normally, no sodium-dependent tail currents were apparent when the membrane was repolarized, but a few minutes after adding veratrine large and slowly decaying current relaxations were recorded (Fig. 2*b*,*c*). Both the inward tail current and the maintained inward current during depolarization were blocked by TTX. Aconitine is also known to modify sodium channels in nerve membranes[17] but caused no obvious changes in the sodium currents in the oocyte, even at a concentration of 5×10^{-4}M.

Scorpion venom (*Leiurus quinquestriatus*) reduced the maximum peak sodium current evoked by depolarizing pulses and also caused the appearance of a TTX-sensitive current which was maintained throughout the depolarizing pulse. In contrast to veratrine the maintained current caused by scorpion venom was rapidly turned off when the membrane was repolarized. The effects of scorpion venom resembled those seen in frog and squid axons[18,19].

Although large (~1 μA) sodium currents could be recorded from oocytes injected with human cortex mRNA it was not normally possible to elicit action potentials by injecting depolarizing current pulses. This was mainly because inactivation of the sodium current was rapid compared with the electrical time constant of the oocyte. However, when inactivation was reduced by veratrine, it became possible to elicit regenerative responses which were blocked by TTX (Fig. 2*d*).

Serotonin receptor channels

Application of serotonin to oocytes injected with human cortex mRNA elicited membrane currents, whilst control oocytes from the same donors showed no responses, even with high drug concentrations (Table 1). Striking features of the serotonin responses were the long latency to onset, and the slow oscillatory

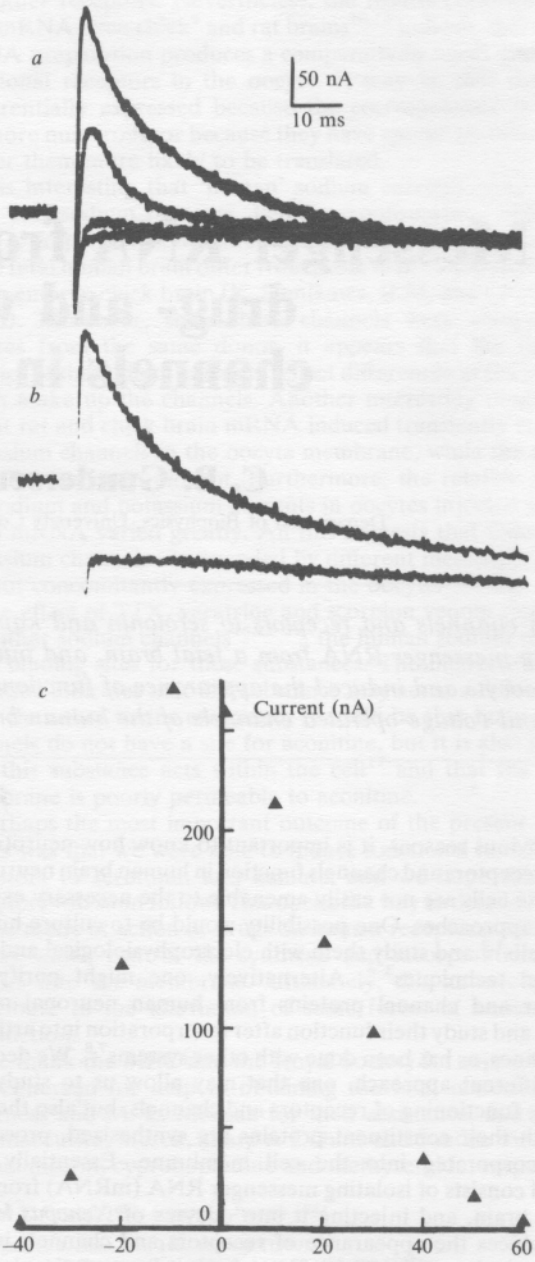

Fig. 1 Voltage dependence of the inward sodium current, in an oocyte injected with human cerebral cortex mRNA. The holding potential was −100 mV in all cases. Temperature, 15.5 °C. *a*, Membrane currents elicited by depolarization to potentials of −50, −30, −20 and −10 mV. The response increased as the potential was made more positive. *b*, Currents elicited from the same oocyte at potentials of +10 and +40 mV. The inward sodium current was smaller at +10 mV than at −10 mV, and was greatly reduced at +40 mV. *c*, Current–voltage relationship for the peak amplitude of the sodium current, measured in the same oocyte. Measurements were made by subtracting the currents elicited at each voltage before and after addition of TTX (313 nM) to the perfusion solution.

Methods: Procedures for isolation of poly(A)$^+$ mRNA and its injection into oocytes of *X. laevis*, were as described previously[10–12,30]. Rat brain mRNA was obtained from Wistar rats, and human brain mRNA was isolated from the cerebral cortex of a 15-week-old fetus. Oocytes were voltage clamped and perfused with Ringer solution at 11–15 °C (refs 9–12). Most experiments were made on oocytes which had been treated with collagenase to remove follicular and other enveloping cells[21].

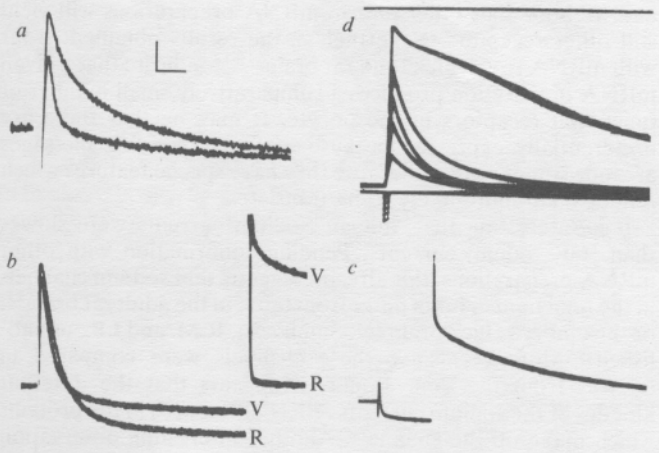

Fig. 2 Effects of repeated stimulation and of veratrine on the decay of the sodium current in oocytes injected with human cerebral cortex mRNA. *a*, Superimposed records showing membrane currents elicited by a pair of depolarizing pulses to −10 mV, from a holding potential of −100 mV. Pulse duration was 900 ms and the pulses were separated by an interval of 100 ms. The oocyte had been injected with human cortex mRNA and was treated with collagenase. Temperature, 15 °C. Calibration bars, 50 nA and 10 ms. *b, c*, Effects of veratrine on the sodium current elicited by a depolarizing pulse to 0 mV. Calibration bars are 50 nA and 50 ms in *b* and 100 nA and 200 ms in *c*. Traces marked R and V in *b* were obtained respectively before and after adding veratrine (0.5 mg ml^{-1}), whilst the trace in *c* shows the tail current in the presence of veratrine on a slower sweep speed. *d*, Action potential elicited in the presence of veratrine from the same oocyte as in *b, c*. Traces show (from top to bottom) 0 mV potential reference; membrane potential; and current injected into the oocyte. A steady current was passed to hold the membrane potential at about −100 mV, and responses are shown to a series of depolarizing current pulses of increasing intensity. The largest pulse triggered a graded regenerative response. Calibration bars, 20 mV (membrane potential), 500 nA (current monitor) and 500 ms. Temperature in *b–d* was 12 °C.

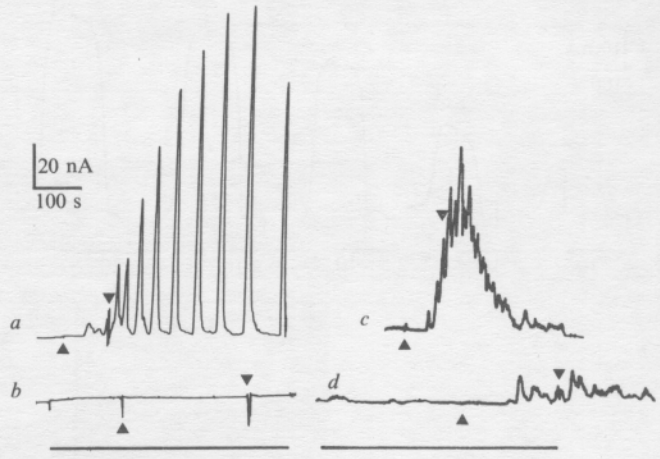

Fig. 3 Oscillatory membrane currents elicited by serotonin in oocytes injected with mRNA from human cerebral cortex. All records were obtained with the oocytes clamped at a potential of −60 mV so as to be away from the equilibrium potentials for sodium, potassium and chloride ions[21]. Serotonin was applied by bath perfusion, beginning and ending as indicated by the arrows. *a*, Response to serotonin recorded from an oocyte in normal Ringer's solution. *b*, Lack of response when serotonin was applied in the presence of methysergide. The bar indicates the duration of methysergide application. The concentration of serotonin was 10^{-6} M in both records, and the concentration of methysergide was 10^{-5} M. Temperature 14 °C. *c, d*, Reduction of size of the serotonin response by lysergic acid diethylamide. Serotonin was applied at a concentration of 5×10^{-7} M in both records, and lysergic acid diethylamide was added at a concentration of 2×10^{-6} M for the time indicated by the bar in *d*. Temperature, 11 °C.

nature of the currents (Fig. 3). In these respects, the responses are quite different from the smooth currents elicited by activation of γ-aminobutyric acid (GABA), kainate or nicotinic acetylcholine (ACh) receptors incorporated into oocytes following injections of the appropriate mRNA[9,13,20]. They do, however, resemble the oscillatory currents elicited by serotonin in oocytes injected with rat brain mRNA[10], as well as the currents elicited by activation of muscarinic ACh receptors[21] and glutamate receptors[12]. In the present experiments, application of ACh and glutamate to oocytes, which showed good sensitivity to serotonin following injection of human mRNA, did not elicit any appreciable currents. Thus, the serotonin responses probably involve a specific serotonin receptor.

The serotonin response was abolished by low concentrations ($<10^{-6}$M) of methysergide, a serotonin receptor antagonist[22] (Fig. 3*a, b*). The blocking action of methysergide appeared to be either irreversible, or only slowly reversible, since responses to serotonin were still blocked even after washing for an hour or more.

In contrast to the powerful blocking action of methysergide, the serotonin antagonists cyproheptadine and ketanserin[23] did not appreciably reduce the size of the response to 10^{-5} M serotonin when applied at a concentration of 10^{-6} M. Lysergic acid diethylamide (2×10^{-6} M) reduced, but did not completely abolish, the response to 5×10^{-7} M setotonin (Fig. 3*c, d*).

Membrane potential and serotonin current

The oscillatory currents induced by serotonin in oocytes injected with human cortex mRNA decreased in size as the membrane was depolarized and inverted direction at a potential of about −20 mV, which corresponds to the chloride equilibrium potential in *Xenopus* oocytes[21]. The equilibrium potential of the serotonin currents in oocytes injected with rat brain mRNA is also similar[10]. Thus, chloride is the main ion flowing through the channels activated by serotonin.

Depolarization beyond the equilibrium potential gave larger serotonin currents than corresponding steps in a hyperpolarizing direction, similar to the rectification seen with serotonin currents induced by rat brain mRNA[10]. This effect probably arose as a result of a voltage-dependent closing of the serotonin-activated channels, since a hyperpolarizing step applied near the peak of a serotonin-activated oscillation initially gave an increase in membrane current, which subsequently declined over a time course of a few hundred milliseconds.

Kainate receptor channels

Application of kainate to oocytes injected with human cerebral cortex mRNA elicited inward membrane currents (Fig. 4). These responses did not show oscillations, and were well maintained during kainate applications lasting several minutes. We did not observe clear responses to glutamate, even when applied at high concentrations (10^{-2} M) to oocytes which had a high sensitivity to kainate. This shows that in humans, as in rats[12], there is a kainate receptor which is distinct from glutamate receptors.

Currents elicited by different doses of bath-applied kainate are illustrated in Fig. 4. The minimal concentration required to give detectable responses was higher than for serotonin. For example, the oocyte illustrated in Fig. 4 gave a just detectable response at 10^{-5} M kainate, while a concentration of 5×10^{-4} M gave a maximal response. At low doses of kainate, a doubling in the concentration more than doubled the peak size of the response.

The reversal potential of the kainate-induced current (estimated by stepping the membrane to different potentials before and during steady kainate application) was about −10 mV. At voltages more negative than the equilibrium potential, the cur-

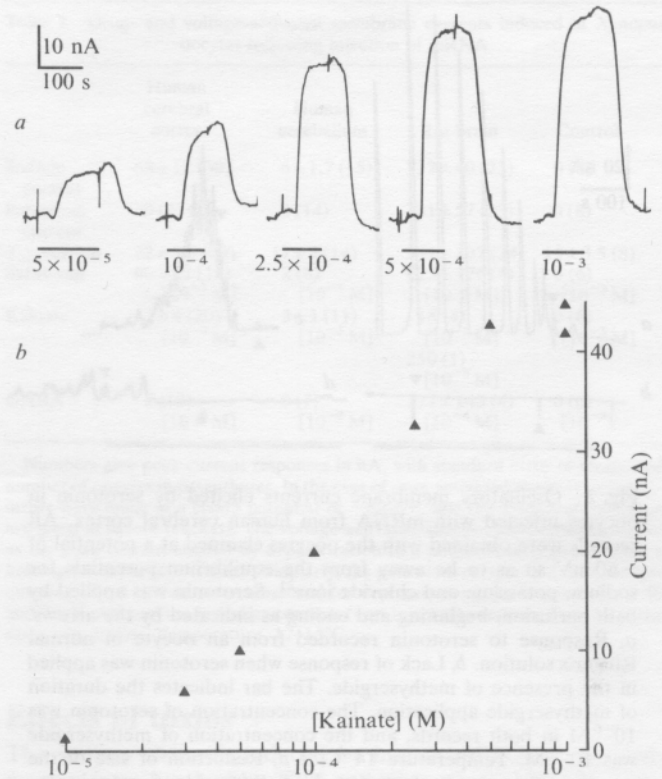

Fig. 4 Membrane currents elicited by kainate in an oocyte previously injected with human cortex mRNA. All records were obtained at a clamp potential of −60 mV. Temperature, 14 °C. *a*, Responses to different concentrations of kainate, applied for the durations indicated by the bars. *b*, Dose/response curve for kainate, measured from the same oocyte as *a*. The peak current amplitude is plotted against kainate concentration on a semi-logarithmic scale.

rent–voltage relationship of the kainate-induced current was close to linear, which is similar to that of the kainate receptor induced by rat brain mRNA[12].

Human and rat membrane channels

Oocytes from two donors were injected with three different mRNA preparations, derived from fetal human cerebral and cerebellar cortices, and from rat brain. The extent to which these particular messenger preparations led to the expression of functional drug- and voltage-activated membrane channels differed markedly (Table 1).

Control (non-injected) oocytes showed no appreciable drug- or voltage-activated responses, except for a small transient outward current[24]. In contrast, oocytes injected with rat brain mRNA gave large responses to serotonin, kainate and GABA and showed large voltage-activated sodium, potassium and transient outward currents (see also refs 10–12). The mRNA derived from human cerebral cortex was less effective. Responses to serotonin and kainate were present, as was the voltage-activated sodium current, but all were smaller than that found with rat brain mRNA. Furthermore, there were no detectable voltage-activated potassium and transient outward currents, nor were there any responses to GABA (Table 1) or to several other putative transmitter substances tested. Oocytes injected with mRNA from fetal human cerebellum showed little development of drug- or voltage-activated membrane currents.

Conclusions

Our results show that mRNA coding for proteins that make up voltage- and drug-operated channels in the human brain, can be effectively translated in *Xenopus* oocytes. Clearly, the cells in the brain contain many types of receptors and channels and we have so far managed to 'transplant' into the oocyte membrane only a few. Perhaps others were present and were not discovered and we hope that other human mRNA preparations will yield still other receptors. Nevertheless, the results obtained so far with mRNA from chick[9] and rat brains[10,12] indicate that a given mRNA preparation produces a comparatively small number of functional receptors in the oocyte. It may be that these are preferentially expressed because the corresponding messages are more numerous, or because they have special features which render them more likely to be translated.

It is interesting that 'human' sodium currents were slower than 'rat' sodium currents. Pending confirmation with other mRNA preparations, this already suggests that sodium channels in the fetal human brain differ from those in the adult rat brain[11], or the embryo chick brain (K. Sumikawa, R.M. and I.P., unpublished). Moreover, since these channels were compared in oocytes from the same donor, it appears that the different kinetics of the sodium currents reflect differences in the proteins which make up the channels. Another interesting observation is that rat and chick brain mRNA induced transiently activated potassium channels in the oocyte membrane, while the mRNA from human brain did not. Furthermore, the relative sizes of the sodium and potassium currents in oocytes injected with rat brain mRNA varied greatly. All this suggests that sodium and potassium channels are encoded by different messengers which are not concomitantly expressed in the oocyte.

The effect of TTX, veratrine and scorpion venom show that, like other sodium channels[16,25–29], the human sodium channels have binding sites for these substances. The ineffectiveness of aconitine indicates that this substance may act at a different site from the other toxins examined. It could be that human brain channels do not have a site for aconitine, but it is also possible that this substance acts within the cell[17] and that the oocyte membrane is poorly permeable to aconitine.

Perhaps the most important outcome of the present experiments was that we were able to induce functional human brain receptors to serotonin and kainate, and we hope that other receptors will soon follow. This opens the way to detailed studies on the mode of action of drugs on human receptors, and on the factors which control the synthesis and incorporation of receptors into the membrane. Ultimately, this knowledge may contribute to the alleviation of some forms of human brain dysfunction.

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