

## Messenger RNA from rat brain induces noradrenaline and dopamine receptors in *Xenopus* oocytes

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*Xenopus* oocytes were induced to acquire sensitivity to noradrenaline and dopamine, by injecting them with poly(A)<sup>+</sup> mRNA isolated from rat brain. In mRNA-injected oocytes, both neurotransmitters elicited a smooth inward membrane current on which was superimposed an oscillatory inward current, which was carried mainly by chloride ions. This contrasts with the native responses that are sometimes seen in non-injected oocytes, where noradrenaline and dopamine both elicit smooth outward currents that are carried mainly by potassium ions. The serotonin antagonist methysergide blocked the induced responses to both noradrenaline and dopamine, and the noradrenaline response was blocked by propranolol.

### INTRODUCTION

Signal transmission across the myriads of chemical synapses in the brain is mediated by the presynaptic release of transmitter substances that act on appropriate receptors located in the postsynaptic nerve cells. At some stage during development, these cells must contain the messenger ribonucleic acids (mRNAs) that code for the receptor proteins. However, it was not known until recently whether nerve cells still contained significant amounts of receptor messengers in the adult brain. We have already shown that the brains of various species, including human, contain mRNAs that code for transmitter receptors; and that these messengers are translated in frog oocytes and cause the formation of functional receptors in the oocyte membrane. By using this approach we have previously induced oocytes to acquire receptors to  $\gamma$ -aminobutyric acid, serotonin, acetylcholine (muscarinic) and several other neurotransmitters (Miledi *et al.* 1982, 1983; Gundersen *et al.* 1983, 1984*a, b, c, d*; Sumikawa *et al.* 1984*a, b*; Parker *et al.* 1984), but not receptors to noradrenaline or dopamine. Here, we report that these can also be induced by poly(A)<sup>+</sup> mRNA isolated from the adult rat brain.

### METHODS

Oocytes of *Xenopus laevis* were injected with about 30 ng of poly(A)<sup>+</sup> mRNA extracted from the brain of adult Wistar rats. The procedures for isolation of mRNA, its injection into oocytes, and for electrophysiological recording were as

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described earlier (Miledi 1982; Miledi *et al.* 1982; Miledi & Sumikawa 1982). However, in the present experiments we used mainly fractions of mRNA which were obtained by sucrose density centrifugation of whole mRNA. We have recently found that appropriate fractions of mRNA are more effective in inducing transmitter receptors and voltage-operated channels, than the whole mRNA from which the fractions derive (Sumikawa *et al.* 1984*a, b*). Results presented here were obtained from oocytes injected with rat brain mRNA fractions 10 or 11, from the same sucrose gradient preparation as described in Sumikawa *et al.* (1984*b*). Drugs were obtained from the Sigma Chemical Company, except for methysergide, which was a gift from Sandoz.

## RESULTS

### *Catecholamine responses in native and injected oocytes*

Kusano *et al.* (1977, 1982) have shown that the membrane of some *Xenopus* oocytes contains receptors to noradrenaline and dopamine. When these receptors are activated by their respective agonists, they give smooth outward membrane currents (for example, figure 1*b*), due to the opening of membrane channels that are permeable mainly to potassium ions (Kusano *et al.* 1977, 1982; Van Renterghem *et al.* 1984). The type and number of transmitter receptors present in the membrane of native oocytes is very variable, and some donors yield oocytes that are insensitive to one or more transmitter substances. For the present experiments we used oocytes that did not give 'native' responses to noradrenaline, dopamine, serotonin and acetylcholine.

In contrast to the lack of responses in these non-injected oocytes, oocytes injected with appropriate fractions of rat brain mRNA responded to bath application of noradrenaline and dopamine; and similar responses were seen occasionally in oocytes injected with the whole mRNA from which the fractions derived. The membrane currents elicited by noradrenaline and dopamine were inward at a membrane potential of  $-60$  mV, and showed marked oscillations (figures 1*a* and 2*a*). They are thus clearly different, both in time course and ionic basis, from the native responses to these drugs, as can be seen by comparing figure 1*a* and *b*.

Another important difference between native and induced catecholamine responses is that in non-injected oocytes the responses disappear when the follicular and epithelial cells, which normally surround the oocyte proper, are removed manually or by collagenase treatment (Kusano *et al.* 1982; Van Renterghem *et al.* 1984; R. Woodward and R. Miledi, unpublished). On the other hand, in oocytes injected with rat brain mRNA the responses to noradrenaline and dopamine persisted after collagenase treatment of the oocytes; as was the case for the oocytes used to obtain figures 1*a*, 2 and 3*a, b*.

It is interesting that some oocytes responded to both noradrenaline and dopamine, while others responded to only one of these substances. This suggests that the receptors are induced by different mRNAs even though, as mentioned below, the responses to both substances are very similar.

We have also observed similar oscillatory responses to noradrenaline in oocytes

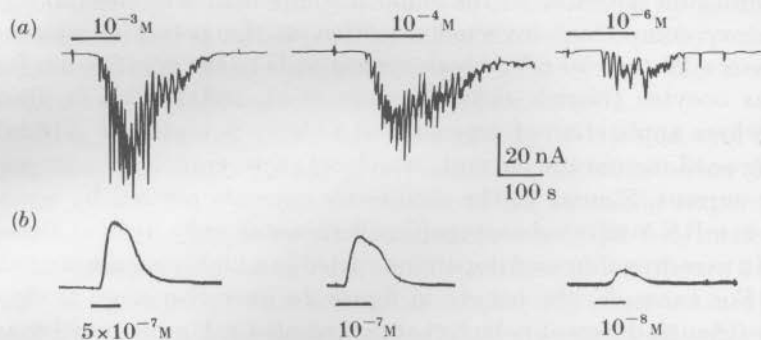


FIGURE 1. Noradrenaline sensitivity induced by rat brain mRNA. The records in (a) show membrane currents elicited by three different concentrations of noradrenaline, applied to an oocyte previously injected with rat brain mRNA fraction 11. For comparison, the records in (b) show responses to noradrenaline in a non-injected oocyte from a different donor. All records were obtained at a clamp potential of  $-60$  mV, and in this, and other figures, downward deflections of the traces correspond to inward membrane currents. Bars indicate the durations of drug applications. The oocyte in (a) was collagenase treated, while that in (b) was untreated. Temperature was about  $20^{\circ}\text{C}$  in all experiments.

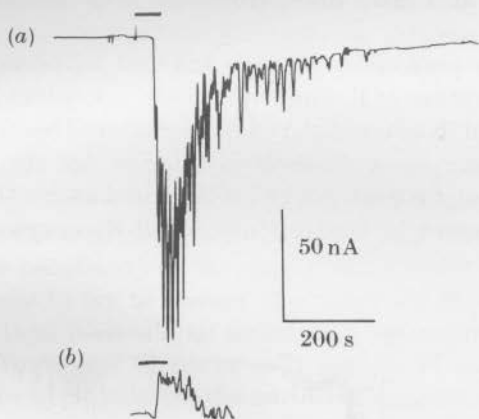


FIGURE 2. Membrane currents elicited by dopamine recorded in two oocytes injected with rat brain mRNA fraction 10. Dopamine ( $10^{-3}$  M) was applied as indicated by the bars, and the oocytes were clamped at potentials of  $-60$  mV (a) and  $-16$  mV (b).

injected with mRNA from cat and rat heart, but these responses were weak and were not seen consistently (C. B. Gundersen, I. Parker & R. Miledi, unpublished data).

#### *Properties of catecholamine responses induced by rat brain mRNA*

The responses to noradrenaline and dopamine in oocytes injected with rat brain mRNA, recorded at a clamp potential of  $-60$  mV, consisted of two components: an initial smooth inward current, on which was superimposed a series of inward current oscillations (figures 1a and 2a). Sometimes both components began at about the same time, but in other cases the oscillations commenced well after the onset of the smooth current, or failed to appear.

The equilibrium potential of the smooth component was close to 0 mV, while the oscillatory component inverted direction as the potential was made more positive than  $-20$  to  $-30$  mV, which corresponds to the equilibrium for chloride in *Xenopus* oocytes (Barish 1983; Kusano *et al.* 1982). This is illustrated in figure 2*b*, where application of dopamine at a clamp potential of  $-16$  mV elicited an initial inward membrane current, which was interrupted by a larger outward oscillatory current. Similar to the oscillatory currents elicited by serotonin and glutamate in mRNA-injected oocytes (Gundersen *et al.* 1983, 1984*a*), the oscillatory responses to noradrenaline and dopamine varied in a highly nonlinear manner with potential. For example, the oocyte in figure 2*a* gave responses to dopamine of similar size (though different polarity) at potentials 4 mV more positive and 40 mV more negative than the equilibrium potential. Hyperpolarizing pulses, given during prolonged application of dopamine and noradrenaline elicited a transient inward membrane current, resembling that seen during activation of serotonin, glutamate and muscarinic receptors (Parker *et al.* 1984).

*Some pharmacological properties of the noradrenaline and dopamine receptors*

A full pharmacological study of the induced dopamine and noradrenaline receptors will be done at a later date. However, some initial results are worth mentioning here.

It is known that the potassium currents induced by noradrenaline in native oocytes are due to activation of  $\beta$ -adrenergic receptors, which are blocked by the  $\beta$ -antagonist propranolol (Kusano *et al.* 1982). The induced noradrenaline receptors, which, as described above, open channels to chloride, are also blocked reversibly by propranolol (figure 3*e*). Propranolol by itself elicited a smooth inward membrane current, which was present in control (non-injected) oocytes, as well as those

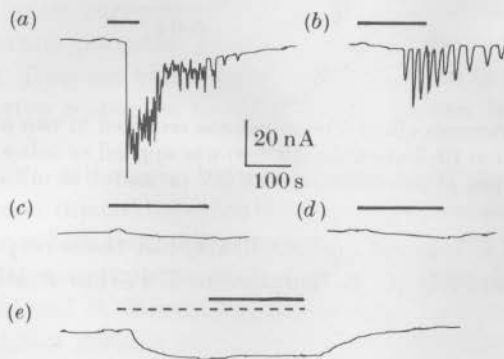


FIGURE 3. Actions of antagonists to noradrenaline and dopamine. (a)–(d) Records from one oocyte, which showed responses to  $10^{-3}$  M dopamine (a) and  $10^{-3}$  M noradrenaline (b). The oocyte was then perfused with methysergide ( $10^{-6}$  M) for a few minutes, and dopamine (c) and noradrenaline (d) were applied at the same concentrations, in the continued presence of methysergide. (e) Record from a different oocyte, perfused with propranolol ( $10^{-3}$  M) as indicated by the dashed line, and noradrenaline ( $10^{-3}$  M) as shown by the solid bar. The trace in figure 2*a* was obtained from this oocyte, before exposure to propranolol. All records at a clamp potential of  $-60$  mV.

injected with mRNA (figure 3*e*). The response was accompanied by an increase in membrane resistance, and inverted polarity when the oocyte was hyperpolarized beyond about  $-100$  mV. Thus, propranolol probably acts to block a resting potassium conductance in the membrane of the native oocyte.

The responses to noradrenaline and dopamine in oocytes injected with rat brain mRNA are very similar to that elicited by serotonin. We therefore tested the action of methysergide, which is a very potent blocker of the serotonin response in the oocyte (Gundersen *et al.* 1984*a, c*). At a concentration of  $10^{-6}$  M, methysergide blocked the oscillatory responses to both noradrenaline ( $10^{-3}$  M, figure 3*a, c*) and dopamine ( $10^{-3}$  M, figure 3*b, d*). This action was consistently observed in all oocytes examined, and the responses recovered only slightly, or not at all, after washing for several hours.

#### DISCUSSION

In many respects the responses to noradrenaline and dopamine resemble those elicited by serotonin, glutamate and acetylcholine in oocytes injected with rat or human brain mRNA (Gundersen *et al.* 1983, 1984*a, c*; Parker *et al.* 1984); but in spite of these similarities, the various responses can be blocked by different antagonists and can be induced independently by different brain mRNA preparations. Thus, it appears that we have a number of different receptors, all of which activate a common system that leads to the opening of chloride channels in an oscillatory fashion. Although noradrenaline, dopamine, serotonin, glutamate and acetylcholine all induced oscillatory chloride currents in *Xenopus* oocytes we do not yet know if similar currents are evoked by neurotransmitters in the cells of the brain from which the mRNA derived. Alternatively, the oscillatory character could depend on some peculiarity of the oocyte. We are now injecting the mRNAs into other types of cells to try to answer this question. If such chloride currents do exist in neurons, then it should be noted that, because of the more negative chloride equilibrium potential in nerve cells compared with the oocyte, these currents would tend to hyperpolarize the nerve cells, rather than depolarize them as happens in the oocyte.

An interesting finding in these experiments was that methysergide blocked the responses to dopamine and noradrenaline. Methysergide is usually classed as a serotonin antagonist and, indeed, we have previously reported that it is a very potent blocker of the serotonin receptors induced in the oocyte by mRNA from rat and human brain (Gundersen *et al.* 1984*a, c*). Thus, it seems that 'serotonin antagonists' used frequently in radioligand binding studies may also be binding to noradrenaline and dopamine receptors. Our results show also that methysergide may be a very useful aid in future studies on the molecular nature of these receptors. In the meantime, it is clear that the adult rat brain contains mRNAs coding for noradrenaline and dopamine receptors, and that we can now add noradrenaline and dopamine to the large list of neurotransmitter receptors that can be functionally expressed in *Xenopus* oocytes.

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