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Serotonin receptors induced by exogenous messenger RNA in *Xenopus* oocytes

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When poly(A)$^+$-mRNA, extracted from rat brain, was injected into *Xenopus laevis* oocytes, it induced the appearance of serotonin receptors in the oocyte membrane. Application of serotonin to injected oocytes elicited, after a long delay, oscillations in membrane current. The equilibrium potential of this current corresponded with the chloride equilibrium potential.

It appears that rat brain mRNA encodes the translation of serotonin receptors into the oocyte membrane. The combination of serotonin with these receptors leads to the opening of membrane channels.

Introduction

Transmission of signals across a myriad of nerve cells in the nervous system is mediated by transmitter substances, which are released from presynaptic nerve terminals and act on receptors embedded in the postsynaptic membrane. Because of the small size, and location, of nerve cells in the central nervous system, it has been difficult to study in detail the ways in which their transmitter–receptor systems operate. Such a task would be considerably simplified if the receptors could be induced to appear in cells more amenable to experimentation. This has been done recently with the γ-aminobutyric acid (GABA) receptors of the chicken brain, which were synthesized in *Xenopus laevis* oocytes and incorporated into their membrane (Miledi *et al.* 1982*b*). The present experiments show that, by injecting messenger RNA (mRNA) extracted from rat brain, we can induce the membrane of *Xenopus* oocytes to acquire receptors to 5-hydroxytryptamine (5HT, serotonin).

Methods

Poly(A)$^+$-mRNA was obtained from the brains of Wistar rats (150–200 g) by means of the chloroform–phenol extraction procedure and enrichment by oligo-dT cellulose chromatography, as described by Miledi & Sumikawa (1982) for cat muscle mRNA. *Xenopus laevis* oocytes were injected with 25–75 ng of poly(A)$^+$-mRNA in HEPES buffer (5 mM, pH 7.5) and kept in Barths medium at 14 °C until used for experiments. In some experiments, oocytes were treated with collagenase (type I and C2139; Sigma Chemical Company) to remove the follicular cells (Kusano *et al.* 1982). Oocytes were voltage-clamped using a two-electrode system, and were continuously perfused with Ringer solution at room temperature

(20–25 °C) during recording. Normal Ringer solution contained (115 mM NaCl, 1.8 mM $CaCl_2$, 2 mM KCl and 5 mM HEPES, at pH 7–7.2. Serotonin (5-hydroxytryptamine, creatinine sulphate complex; Sigma Chemical Company) was applied either by bath perfusion or by iontophoresis from a micropipette filled with a saturated solution in water. For further details of methods see Kusano *et al.* (1982), Miledi & Sumikawa (1982) and Miledi (1982).

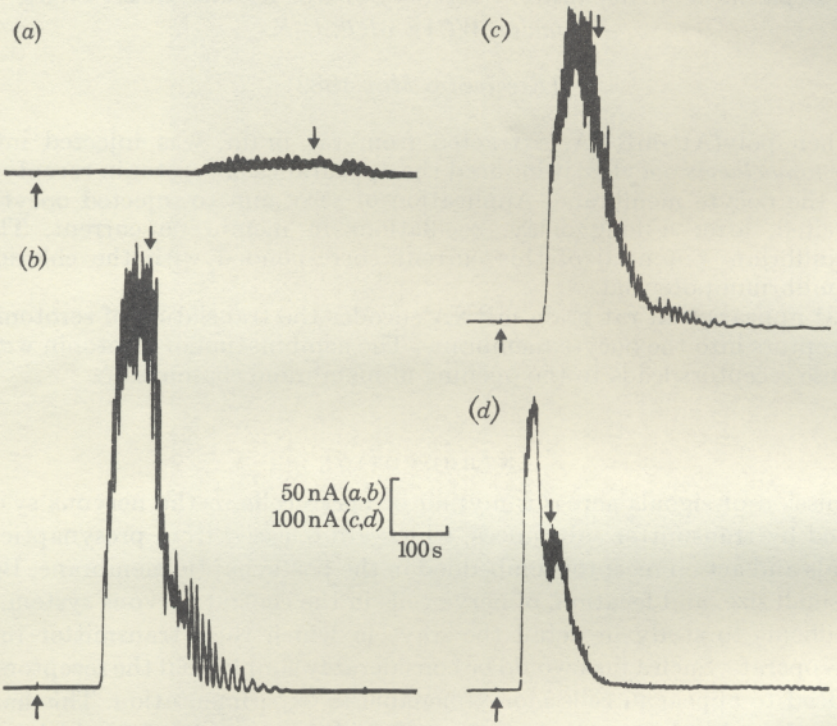

FIGURE 1. Membrane currents elicited by different concentrations of serotonin applied by bath perfusion to a collagenase-treated (c.t.) oocyte that had previously been injected with rat brain mRNA. Application of serotonin began and ended at the times indicated by the arrows. All traces were obtained with the oocyte clamped at a potential of −60 mV. Upward deflexions in this, and other figures, denote inward membrane current. Concentrations of serotonin used were: (*a*) 2×10^{-8} M, (*b*) 5×10^{-8} M, (*c*) 10^{-7} M, (*d*) 10^{-5} M.

RESULTS

Induction of serotonin sensitivity

When serotonin was applied to oocytes that had been injected a few days earlier with rat brain mRNA, membrane current responses were observed (figure 1). Small responses to serotonin are occasionally observed from non-injected oocytes (Kusano *et al.* 1982), but in the present experiments these responses were clearly induced as a result of the injection of mRNA, since control oocytes (non-injected, or injected with HEPES buffer) from the same donors showed no sensitivity to serotonin. Many control oocytes were examined, none of which had responses to serotonin. In contrast, practically all oocytes derived from several donors and injected with rat brain mRNA showed responses.

The oocytes injected with rat brain mRNA still responded to serotonin after the follicular and other enveloping cells were removed by collagenase treatment. This indicates that the serotonin sensitivity resides in the oocyte membrane itself and, incidentally, demonstrates that the collagenase treatment used does not destroy the serotonin receptors. Furthermore, when serotonin was applied iontophoretically to the oocyte surface, It was found that both the animal and the vegetal hemispheres had acquired serotonin sensitivity.

The induction of serotonin sensitivity did not appear to be caused by activation of the oocytes' own genome, because the oocytes became sensitive to serotonin even when they were exposed continuously to actinomycin D (10 μg ml^{-1}), to prevent synthesis of mRNA. Nor can the induction of serotonin sensitivity be ascribed to the injection *per se*, because oocytes injected with mRNA from the optic lobe of chick embryos did not respond to serotonin (R. Miledi, I. Parker & K. Sumikawa, unpublished data). From all this we conclude that after injection of rat brain mRNA the membrane of the oocyte acquires serotonin receptors and their associated ionic channels, and that this process is caused by the injection of specific messengers.

Properties of serotonin activated currents

A striking feature of the membrane currents elicited by serotonin in oocytes injected with rat brain mRNA was the long latency to onset, and the slow oscillatory nature of the responses (figures 1–3). In these respects, the serotonin responses resemble the slow oscillatory muscarinic acetylcholine (ACh) responses observed in some oocytes (Kusano *et al.* 1982; Miledi *et al.* 1982*a*) but are entirely unlike the fast, smooth, membrane currents elicited by activation of GABA or nicotinic ACh receptors incorporated into the oocyte membrane following injection of appropriate mRNAs (Miledi & Sumikawa 1982; Miledi *et al.* 1982*a*, *b*).

The latency to onset of the response decreased with increasing concentration of serotonin in the perfusion fluid. For example, in the oocyte illustrated in figure 1, a latency of about 280 s was recorded at a concentration of 2×10^{-8} M, and this decreased in a graded fashion with increasing dose, to a latency of about 45 s at a concentration of 10^{-5} M. Much of this latency at high concentrations would have been due to dead time in the perfusion system. By using strong iontophoretic pulses of serotonin applied to the oocyte membrane, much shorter latencies were observed (cf. figure 2), and sometimes latencies as short as 2 s could be recorded. In contrast to the graded dose dependence of the latency, the peak current amplitude during a response increased from zero to nearly maximal over a very narrow concentration range. For example, the oocyte in figure 1 gave no response at a concentration of 10^{-8} M, and only a small response at 2×10^{-8} M. However, a concentration of 5×10^{-8} M gave a large response, which increased by only about 40% when the concentration was further raised by a factor of 200, to 10^{-5} M.

Dependence of serotonin activated current on membrane potential

The oscillatory currents induced by serotonin decreased in size as the membrane was depolarized and inverted direction at about −24 mV (figure 2), which corresponds to the chloride equilibrium potential in *Xenopus* oocytes (Kusano *et al.* 1982). The spontaneous fluctuations in membrane potential and the oscillatory

muscarinic response to ACh, both of which are caused by increases in chloride conductance (Kusano *et al.* 1982), reverse at about the same potential as the serotonin response. Also, the reversal potential was found to be altered by changes in chloride concentration in the Ringer solution, but was little affected by removal of sodium ions. All this indicates that chloride is the main ion permeating the membrane channels that are activated by serotonin.

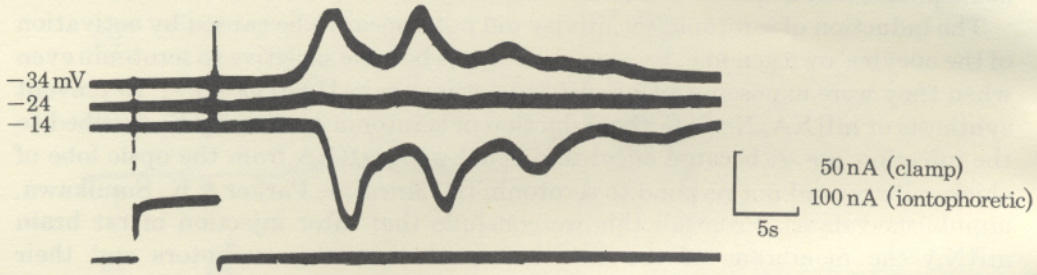

FIGURE 2. Reversal potential of the current oscillations induced by serotonin. Three traces are shown of membrane current recorded at different potentials in response to a constant iontophoretic pulse of serotonin. The upper three traces are membrane currents recorded at potentials of (from top to bottom) −34, −24 and −14 mV. The lowest trace shows iontophoretic current through the serotonin pipette. Oocyte injected with rat brain mRNA and treated with collagenase.

When the membrane potential was made progressively more positive than the equilibrium potential, the serotonin-induced currents increased strongly. However, corresponding shifts in a negative direction from the equilibrium potential gave smaller increases in response, and at hyperpolarized potentials the response decreased as the voltage was made more negative, in spite of the increased driving force for chloride efflux. This is illustrated in figure 3, where hyperpolarization from −60 to −140 mV during a serotonin response caused a reduction in the peak-to-peak excursions of the oscillations in current. Also, the mean membrane current showed little change when the membrane was hyperpolarized during serotonin action, even though the same hyperpolarization applied before the response caused an appreciable increase in current. The explanation for this is probably that the increase in 'passive' current caused by the hyperpolarization was closely matched by a decrease in the serotonin-activated current.

Pharmacology

A pharmacological characterization of the serotonin receptors in oocytes injected with rat brain mRNA is in progress and will be described at a later date. Suffice to say here that the response to serotonin was abolished by D-lysergic acid diethylamide (0.5 μg ml^{-1}; Delysid, Sandoz) and by theophylline (5 mM). Adenosine (1 mM) and dopamine (1 mM) strongly reduced the response. Atropine (5×10^{-7} M), curare (10^{-4} M), picrotoxin (1 mM) and strychnine (10^{-4} M) did not reduce the response. Tryptamine elicited oscillatory responses at concentrations as low as 10^{-7} M.

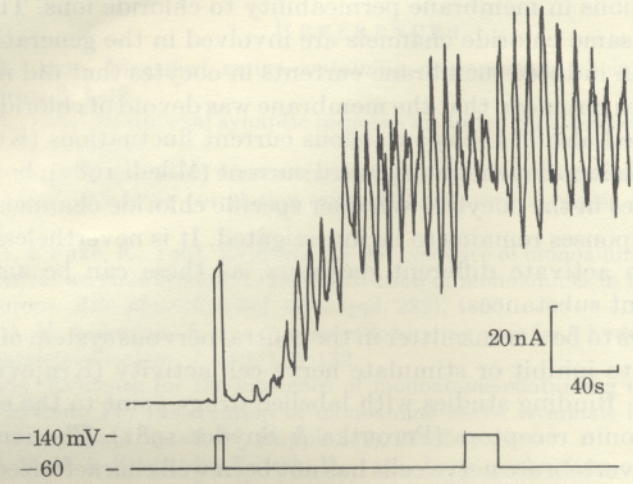

FIGURE 3. Rectification of the serotonin induced current at hyperpolarized potentials. Serotonin (2×10^{-8} M) was applied by bath perfusion throughout the record, beginning a little before the start of the trace. The oocyte was clamped at a potential of −60 mV, except for two pulses to −140 mV as indicated below the record. Note that when the oocyte was hyperpolarized during the serotonin response, the amplitude of the current oscillations decreased and there was little change in mean current, even though the same hyperpolarization applied before the response had built up caused a large passive current. Oocyte injected with rat brain mRNA and treated with collagenase.

DISCUSSION

Our experiments show clearly that *Xenopus* oocytes injected with mRNA extracted from rat brain develop sensitivity to serotonin. But how does the foreign mRNA induce this sensitivity? The most likely possibility is that the rat brain mRNA directs the synthesis of one or more proteins, that convey to the oocyte membrane its serotonin sensitivity. Alternatively, the exogenous mRNA could trigger the transcription of the relevant mRNA from the oocyte's own genome; but this does not seem likely because serotonin sensitivity still developed in oocytes that were exposed to actinomycin-D, before and after the injection of rat brain mRNA. Another possibility is that the foreign mRNA triggers the translation of some of the endogenous mRNA already present, but previously inactive, in the oocyte. However, injection of exogenous mRNA has generally been found to decrease endogenous protein synthesis, except in the case of adenovirus mRNA, which stimulates the synthesis of *Xenopus* oocyte protein (Richter *et al.* 1982). At present we cannot entirely rule out these possibilities, but it seems likely that the rat brain mRNA is translated by the oocyte, and that the processing of the translated products makes the oocyte membrane sensitive to serotonin. Presumably the rat brain mRNA codes for proteins that are incorporated into the membrane and make a receptor–channel complex capable of being activated by serotonin.

The combination of serotonin with the receptors induced by rat brain mRNA evoked responses that were remarkably similar to those elicited by ACh acting on native muscarinic receptors: both responses occurred after a long delay and were

made up of oscillations in membrane permeability to chloride ions. The question arises whether the same chloride channels are involved in the generation of both responses. Serotonin induced membrane currents in oocytes that did not respond to ACh, but this does not mean that the membrane was devoid of chloride channels, as these are involved also in the spontaneous current fluctuations (Kusano *et al.* 1982) and in the calcium-dependent outward current (Miledi 1982), both of which are common features in the oocytes. Whether specific chloride channels are linked to each of these responses remains to be investigated. It is nevertheless clear that serotonin and ACh activate different receptors, as these can be activated, or blocked, by different substances.

Serotonin appears to be a transmitter in the central nervous system of mammals, where it is known to inhibit or stimulate nerve cell activity (Krnjević & Phillis 1963; Couch 1970). Binding studies with labelled drugs point to the existence of two types of serotonin receptors (Peroutka & Snyder 1981). The ionic basis of serotonin action in vertebrate nerve cells has not been well characterized, although in nerve cells of the rat hippocampus, serotonin activates potassium channels (Segal 1980). In contrast, serotonin receptors in molluscan neurons have been better studied and six, or more, types of response to serotonin have been described (Gerschenfeld & Paupardin-Tritsch 1974; Cottrell 1977; Pellmar & Carpenter 1980). The serotonin responses in oocytes injected with rat brain mRNA are unlike any previously described. The one that resembles it most is a calcium-dependent, oscillatory response to serotonin seen in some nerve cells of the snail (Cottrell 1981).

The cellular origin of the rat brain mRNA that codes for the serotonin receptors induced in the oocytes is not known. We presume that the messengers derive from serotonin-containing neurons (Dahlström & Fuxe 1965), which are themselves sensitive to serotonin (Haigler & Aghajanian 1974), and from the postsynaptic neurons contacted by their projections (Fuxe 1965). However, we are also examining the possibility that the relevant mRNA is not of neuronal origin, but derives from vascular elements. In particular, the platelets are capable of binding, taking up, storing and releasing serotonin (Sneddon 1973; Drummond & Gordon 1975).

Whatever the source of the mRNA turns out to be, it is very surprising that only serotonin receptors were induced in the oocyte when, presumably the rat brain contains messengers coding for many other transmitter receptors. The same problem arose in connection with the mRNA extracted from chick brains, which coded for GABA receptors (Miledi *et al.* 1982). This is probably a very important problem, whose eventual answer may give some clues on the processes by which different types of nerve cells develop. To explore this question it will be helpful to isolate specific messengers. In the meantime, by using crude mRNA fractions, we can profitably go on 'fishing' for receptors, because once they are induced to appear in the oocyte they can be studied in great detail.

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