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Intracellular Ca^{2+}-dependent and Ca^{2+}-independent responses of rat brain serotonin receptors transplanted to *Xenopus* oocytes

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SUMMARY

Xenopus oocytes injected with messenger RNA extracted from rat brain are induced to acquire a variety of neurotransmitter receptors and voltage-operated membrane channels. Activation of the receptors to serotonin, acetylcholine (muscarinic) and glutamate elicits oscillatory membrane currents carried by chloride ions. These currents are not abolished by removing external calcium, but are completely abolished after EGTA is injected into the oocytes to chelate intracellular calcium. A smooth current response to serotonin remained in EGTA-loaded oocytes, indicating that this response does not require intracellular calcium. In contrast to the oscillatory chloride currents, the chloride currents activated by GABA or glycine are not abolished by intracellular injection of EGTA. Thus, there appear to be two classes of chloride channels one of which requires intracellular calcium to open.

Some oocytes of the frog, *Xenopus laevis*, have cholinergic and catecholaminergic receptors in their membrane[2,8,9], although, some donors yield oocytes that do not have receptors. When oocytes are injected with poly(A)$^+$ messenger RNA (mRNA) extracted from the brain, the foreign mRNA is translated and the oocytes acquire functional receptors to neurotransmitters. Using this procedure it has been possible to 'transplant' many neurotransmitter receptors from brain cells into the oocyte membrane[3-7,13,16,17]. For instance, rat or human brain mRNAs cause the oocytes to acquire serotonin receptors, whose activation elicits oscillatory membrane currents due to successive

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opening and closing of chloride channels[3]. These currents resemble those seen after activation of muscarinic acetylcholine receptors, and in both cases it was suggested that the opening of the chloride channels was triggered, not directly by the binding of the transmitter to the receptor, but indirectly by the action of an internal messenger[3,8,9]. This messenger might be calcium ions since these are known to open chloride channels[10,11].

To examine the role of intracellular calcium ions in the generation of the serotonin response of oocytes injected with rat brain mRNA (for methods see refs.4,9,10,12), we loaded oocytes intracellularly with the calcium-chelating agent, EGTA (ethylene glycol-bis(β-aminoethyl ether)N,N′-tetraacetic acid). Loading with EGTA was deemed adequate when the transient outward current[10] was abolished because this current, evoked by membrane depolarization, is known to depend on a rise in intracellular calcium[11].

The responses to serotonin in oocytes previously injected with rat or human brain mRNA normally consist of two types of inward membrane currents (at -60 mV): one appears smooth at the electrical amplification used, while the other and much larger one is the oscillatory current already mentioned above. After loading the oocytes with EGTA the oscillatory current was abolished but the smooth component remained (Fig. 1). Abolition of the serotonin-induced oscillations was seen in all oocytes adequately loaded with EGTA, either after pressure injection or ionophoresis[11]. The block of the oscillations was long-lasting and persisted even days after EGTA injection. It is unlikely, therefore, that the effect arose from any artifact of the injection procedure, such as passage of the ionophoretic current or damage during impalement of the injection pipette. Thus, the most likely explanation is that the oscillatory currents are abolished because intracellular calcium ions are chelated by the EGTA.

Normally, the smooth current response to serotonin is difficult to study because it is overwhelmed by the oscillatory component. However, after loading with EGTA, and abolishing the oscillatory currents, the smooth component can be studied in isolation. In the batch of oocytes used, the smooth current response became detectable at serotonin concentrations of ca. 10^{-8} M, and reached a maximum size at between 10^{-7} and 10^{-6} M (Fig. 2). Even at much higher concentrations the current response to serotonin showed no detectable oscillations in oocytes well loaded with EGTA. The smooth current appears to consist of two components, both of which are present in normal mRNA-injected oocytes but are usually obscured by the larger oscillatory current. One component is accompanied by an increase in conductance, and inverts direction at about the chloride equilibrium potential, while the other component shows a decrease in conductance, and probably arises from a closing of potassium channels.

One possible explanation for the blocking action of intracellular EGTA is that the oscillatory response to serotonin depends on an increase in intracellular calcium ions, consequent to calcium entering the oocyte from the external medium. To test this possibility, we bathed oocytes in solutions containing very low levels of free calcium, or including agents such as lanthanum (100 μM), manganese (10 mM) or cobalt (10 mM), all of which are known to block calcium channels. Although these procedures

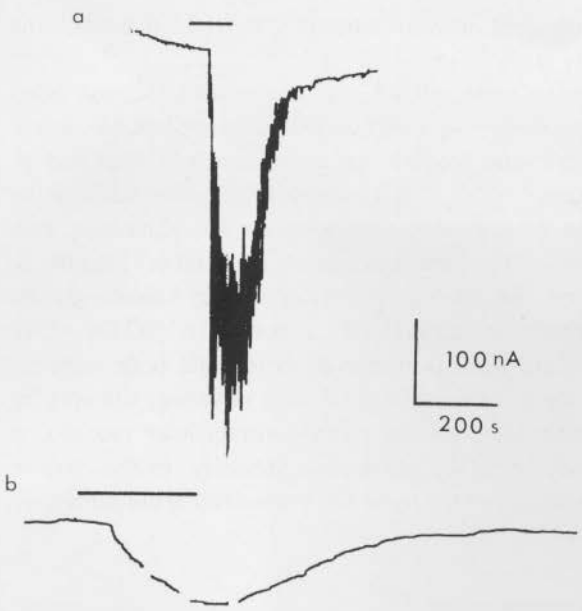

Fig. 1. Abolition of serotonin-induced oscillatory currents, by intracellular loading with EGTA. a: control record from an oocyte injected with rat brain mRNA. Serotonin (3×10^{-8} M) was applied by bath perfusion for the time indicated by the bar. b: record from the same oocyte, but after intracellular loading with EGTA, using ionophoretic pulses of ca. 50 nA for about 1 h. The potential was stepped to different levels during the breaks in the trace. For this and other figures the oocytes, injected with rat brain mRNA, were voltage-clamped at -60 mV and continuously superfused with Ringer solution at about 20 °C. Downward deflexion of the trace corresponds to inward membrane current.

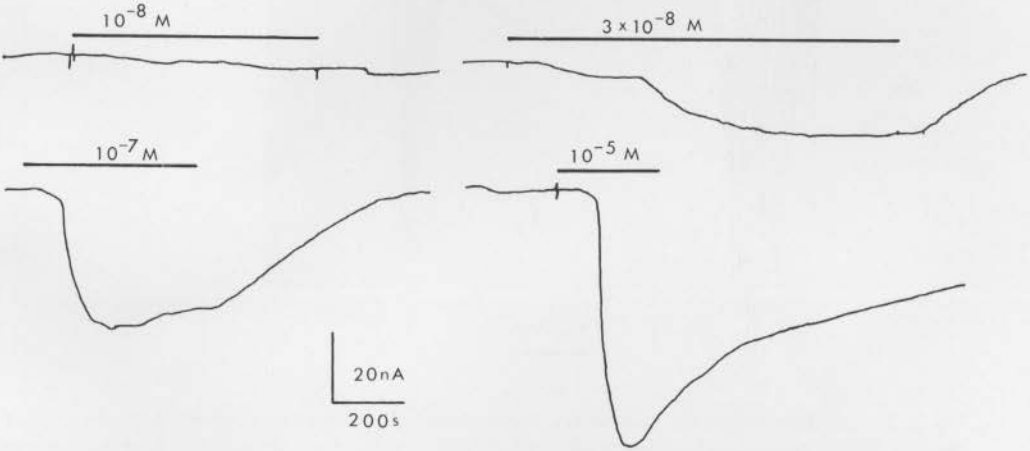

Fig. 2. Responses to different concentrations of serotonin applied to an oocyte after intracellular loading with EGTA had abolished the oscillatory currents.

abolished the calcium-dependent transient outward current[10], they did not block the oscillatory responses to serotonin (Fig. 3).

Oocytes injected with rat or human brain mRNA give responses to several other neurotransmitters. These include oscillatory currents activated by glutamate, acetylcholine, noradrenaline and dopamine; and smooth membrane currents activated by kainate, γ-aminobutyric acid and glycine[4,6,13,16,17]. It is interesting that after intracellular loading with EGTA, the oscillatory responses to acetylcholine and glutamate were abolished, leaving smaller smooth inward membrane currents (at − 60 mV) similar to those seen with serotonin. In contrast, the smooth currents elicited by kainate, glycine and γ-aminobutyric acid were virtually unchanged after injections of EGTA which completely abolished the oscillatory currents. It should be noted that both γ-aminobutyric acid and glycine open chloride membrane channels, but obviously the opening of these drug-operated chloride channels does not require intracellular calcium, in contrast to the chloride channels activated by serotonin. Similarly, in the case of chloride channels opened by membrane depolarization, those involved in the generation

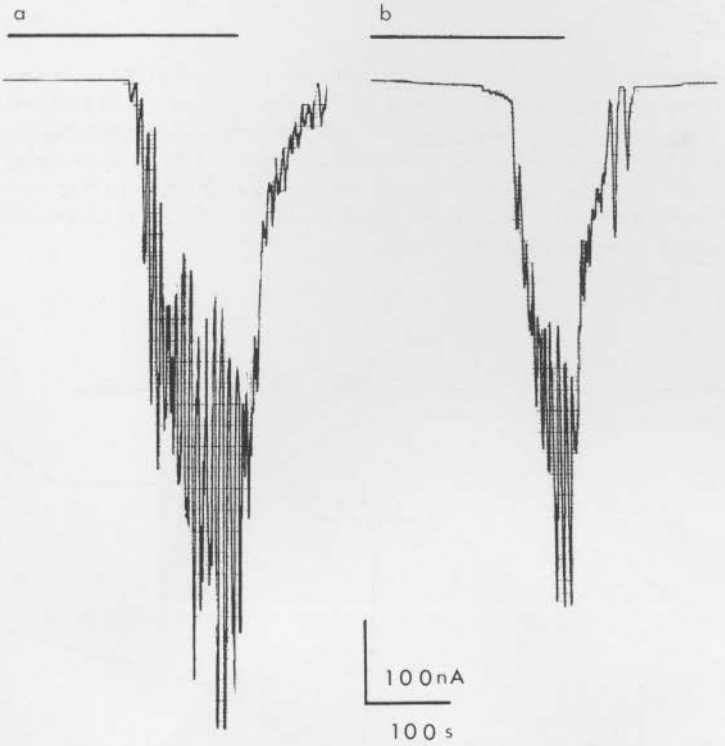

Fig. 3. The oscillatory response to serotonin is not abolished by removal of calcium from the external medium. Both traces show responses to serotonin (5×10^{-7} M) : (a) in normal Ringer; and (b) in Ringer with no added calcium and containing 2 mM EGTA and 5 mM $MgCl_2$. Perfusion with the zero calcium Ringer began several minutes before record b.

of the transient outward current, either native[10] or induced by brain mRNA[11], require intracellular calcium, while the voltage-operated chloride channels induced by mRNA from *Torpedo* electric organ are still able to open in oocytes loaded with EGTA[18].

The oscillatory currents elicited by serotonin, acetylcholine and glutamate in oocytes injected with rat brain mRNA show closely similar properties, even though the receptors involved are pharmacologically different[3,4,14] and are coded by different mRNAs[16]. Thus, these receptors expressed in oocytes, and also receptors for dopamine and noradrenaline[17], all appear to activate a common system that leads to opening of chloride channels in an oscillatory fashion. furthermore, several characteristics of the responses including their long latency of onset and slow time course, suggest that the coupling between transmitter–receptor binding and opening of chloride channels involves intracellular messenger substances[3,8,9]. The present experiments indicate that intracellular calcium ions play a role in this internal messenger system, and show further that an influx of calcium from the external medium is not involved in the generation of the oscillatory membrane currents elicited by serotonin, acetylcholine or glutamate.

Therefore, it appears that activation of serotonin, acetylcholine or glutamate receptors causes the intracellular level of free calcium to be raised and this leads to the opening of calcium-dependent chloride channels. This interpretation is supported by the finding that an influx of calcium, or direct injection of calcium into the oocytes, elicits a chloride current[10,11]. After this paper had been written, it was reported that inositol 1,4,5-trisphosphate mimics the muscarinic response in native *Xenopus* oocytes[15]. It may be that an increase in inositol trisphosphate is also involved in the generation of the responses to serotonin and other neurotransmitters that trigger oscillatory chloride currents in *Xenopus* oocytes, and that this increase causes the release of calcium from intracellular stores as occurs in other systems[1].

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