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Microtransplantation of ligand-gated receptor-channels from fresh or frozen nervous tissue into *Xenopus* oocytes: A potent tool for expanding functional information

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ABSTRACT

Despite huge improvements in neurobiological approaches for investigating the functional properties of neurotransmitter receptors and ion channels, many difficulties are still encountered when focusing on the human brain. Electrophysiological studies aimed at performing direct determinations on human nervous tissue are limited by neurosurgery and also by pathophysiological conditions prevailing before and after the resective operation. The electrophysiological study of receptors and channels becomes difficult also in animal models when the cells are not accessible and/or the experiments last many hours, during which the examined nervous tissue usually becomes unhealthy. To increase the possibility of doing optimal electrophysiological recordings, addressed to investigate the functional properties of receptors and channels, more than two decades ago, foreign mRNAs were injected into *Xenopus* oocytes to heterologously express the receptors; and about a decade ago cell membranes were injected into the oocytes to directly transplant the native receptors. While the first approach needs complex procedures for mRNA isolation, the membrane preparations are simpler to obtain and the embedded receptors are transplanted in their own membrane, with their own glycosylation and together with any ancillary proteins they may have. Using injections of membranes isolated from fresh nervous tissues several issues have already been addressed and many questions can be answered in the near future. Strikingly, with this approach it has been possible to "resuscitate" receptors and ion channels from tissues kept frozen for many years. This review focuses on recently obtained information and on some new lines of biological research using receptor microtransplantation into oocytes.

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Abbreviations: TLE, temporal lobe epilepsy; nAChRs, nicotinic acetylcholine receptors; GluRs, ionotropic glutamate receptors; $GABA_A$, γ-aminobutyric acid type A; CTLE, childhood temporal lobe epilepsy; CD, cortical dysplasia; BDNF, brain-derived neurotrophic factor; AD, Alzheimer's disease.

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1. Introduction

The experimental approach of injecting into *Xenopus* oocytes poly(A+) mRNA isolated from many tissues to express receptor proteins and channels was a revolutionary and powerful technique, first introduced by Miledi and colleagues (Metafora et al., 1976; Barnard et al., 1982), that made it possible to heterologously express, study and compare the properties of the major neurotransmitter receptors and transporters of many tissues and species. Despite the great wealth of information on receptor structure and function that has been obtained during the subsequent decades using mRNA injections into frog oocytes, there are some limitations concerning the complex procedures used to isolate fragile mRNA's with strong expressional potency and also the fact that one is losing the native protein processing as well as the lipids in which the original receptors were embedded. To overcome these problems Miledi and colleagues proceeded to inject oocytes with membranes isolated from the electric organ of *Torpedo*; and this led the oocytes to acquire *Torpedo* functional acetylcholine receptors and voltage-operated chloride channels, still embedded in their original lipid membrane (Marsal et al., 1995). Subsequently, nicotinic acetylcholine receptors (nAChRs) purified from the electric organ and reconstituted in a lipid matrix were used to see whether nAChRs embedded in heterologous lipids could be incorporated correctly in a host oocyte membrane. Proteoliposome injections into the oocytes led to the appearance of functional nAChRs, most of them with the correct orientation (Ivorra et al., 2002), ACh being substantially ineffective if applied inside the oocyte (Morales et al., 1995). The injection of proteoliposomes into *Xenopus* oocytes extends from ligand-gated receptors to transporters such as aquaporins (Le Cahérec et al., 1996; Bossi et al., 2007). We review here the use of the receptor microtransplantation technique focusing on ligand-gated receptor/channels and the results obtained to date, thus paving new roads for further development and applications.

2. Receptor microtransplantation by injecting membranes into *Xenopus* oocytes

2.1. The technical approach in brief

The heterologous expression of ligand-gated and voltage-gated ion channels in the oolemma of *Xenopus* oocytes is considered one of the most powerful tools for determining their function and structure. This experimental approach is particularly useful when the native cells are not easily amenable to extensive investigations, as for instance cells from the human brain. Two methods with comparable efficacies may be used to express receptors and channels in the oocyte membrane: (A) the cytoplasmic injection of *Xenopus* oocytes with poly(A+) mRNAs extracted from native tissues, or intranuclear injection of cDNAs encoding ion channels and (B) the cytoplasmic injections of membrane vesicles isolated from native tissues. The latter method shows some technical advantages compared to the first one:

- Easier preparation of the material to be injected;
- faster functional expression of neurotransmitter receptors compared to the classical mRNA injection;
- more stable preparation because degradation by RNAase is avoided;
- small amounts of tissue required and the same aliquots of membrane preparations can be used after thawing and freezing many times without many precautions.

A sketch of the"microtransplantation" method is shown in Fig. 1, while procedures routinely used are summarized in the following lines (and detailed in Miledi et al., 2002, 2006):

- 0.1–0.5 g of frozen tissue are homogenized in glycine buffer;
- the filtrate is centrifuged for 15 min at 9500 × *g*;
- the supernatant is centrifuged for 2 h at 100,000 × *g* with an ultra-centrifuge;
- the pellet is washed, re-suspended in assay buffer (glycine 5 mM) and used directly or aliquoted and kept at −80 °C for later usage;
- the preparation of *Xenopus laevis* oocytes and injection procedures are fully detailed in Miledi et al. (2002, 2006);
- oocytes are injected with membrane fractions (50–100 nl; 0.2–10 mg protein/ml) dissolved in 5 mM glycine, and maintained in modified Barth's solution plus antibiotics at 16 °C until the electrophysiological recordings are performed.

However, when necessary, tissue samples 10–50 mg were used for membrane preparation.

2.2. Technical improvements of the method

Many attempts have been made to see whether protein concentration in the membrane preparation affects either the receptor expression delay or the density of functional receptors expressed, so far without very conclusive results. Preparations with different protein concentrations exhibit different time courses of receptor incorporation: the more concentrated samples generally incorporating more slowly. Moreover, a critical protein concentration for each preparation exists, above which the amplitude of the currents recorded does not increase further and the injection becomes deleterious to the oocytes. Since the injected membrane vesicles undergo a fusion process with the oolemma, it could be thought that cytosolic Ca^{2+} is involved in the mechanism of membrane fusion. Therefore, increasing the intracellular Ca^{2+} level could improve the incorporation of receptors after membrane injections. However, Morales and colleagues (Gal et al., 2000) showed that the incorporation of proteoliposomes into the *Xenopus* oocyte membrane is not triggered by an intracellular Ca^{2+} increase being a "constitutive mechanism" of oocytes, indicating that Ca^{2+} is not stringently required for the fusion process. The same authors suggested that the mechanism of membrane fusion could be accelerated by protein phosphorylation or by incorporation of fusion proteins with membranes before their injection (Gal et al., 2000). To date, these issues have not been addressed further. A complication of membrane microtransplantation from a nervous tissue to the