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Microtransplantation of human brain receptors into oocytes to tackle key questions in drug discovery

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It is important in drug discovery to demonstrate that activity of novel drugs found by screening on recombinant receptors translates to activity on native human receptors in brain areas affected by disease. In this review, we summarise the development and use of the microtransplantation technique. Native receptors are reconstituted from human brain tissues into oocytes from the frog *Xenopus laevis* where they can be functionally assessed. Oocytes microtransplanted with hippocampal tissue from an epileptic patient were used to demonstrate that new antiepileptic agents act on receptors in diseased tissue. Furthermore, frozen post-mortem human tissues were used to show that drugs are active on receptors in brain areas associated with a disease; but not in areas associated with side effects.

Introduction

Drug discovery for ion channel targets usually involves HTS of compounds on recombinant receptors heterologously expressed in mammalian cell lines [1–3]. This approach is somehow artificial because native ion channels in the brain are mostly multimeric protein complexes where stoichiometry is rarely known. Furthermore, their interactions with accessory subunits and scaffolding proteins is known to influence their functional and pharmacological properties. It is important therefore to evaluate whether compound activity as determined on recombinant channels translates into activity at fully assembled ion channels in their native environment. For this purpose, primary cultured neurons from rodent brain areas are frequently used. However, drugs must ultimately be active at the native human targets, specifically in patients, and therefore it is highly desirable to be able to demonstrate that activities of drugs found in human recombinant and native rodent neurons translate to the human native targets and, where possible, human targets in the diseased state. Primary cultured human neurons are far less frequently used than their rodent counterparts; but in some cases viable human primary neurons or brain slices from tissue biopsies have been used [4–6]. The importance of translational studies between rodent and human neurons is nicely illustrated by the recent finding that, although the right molecular constituents were present in rodent and human preparations, an analgesic mechanism observed in mouse dorsal root ganglia (DRG) neurons did not function in human DRG neurons [7]. For some drug targets, blood cells or tissue biopsies can be used to gain confidence that candidate molecules are active at native human ion channels. Also, human neuroblastoma cell lines [8–10] or human stem-cell-derived neurons [11,12] can be used for some ion channels and receptors. However, these cellular approaches have their own disadvantages, being the peripheral and tumoral origin of neuroblastoma cells or the rather undifferentiated nature of several induced pluripotent stem cell (iPSC)-derived neurons [13]. For these reasons, it is not certain whether ion channels expressed by neuroblastoma or stem-cell-derived neurons properly reflect the molecular assembly of the ion channels in native tissues.

As part of our α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist programme [14], we asked ourselves whether it was possible to use post-mortem human tissues as a means to evaluate whether antagonist activity of compounds at recombinant AMPA receptors translates to antagonist effects at native human receptors. We found a series of papers by Ricardo Miledi and co-workers [15,16], who developed an elegant method that allowed them to characterise the functional properties of native human neurotransmitter receptors microtransplanted from post-mortem human brain into oocytes from

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https://doi.org/10.1016/j.drudis.2018.10.015

the African clawed frog (*Xenopus laevis*). In addition, they also used this approach to characterise the function of receptors obtained from brains of patients suffering from various neurological diseases, such as epilepsy [4,17–23], Alzheimer's disease (AD) [24,25], autism [26,27] and Angelman syndrome [28]. In a few cases, the properties of the receptors obtained from the diseased brains were significantly different from the properties of the same receptors obtained from the same brain regions of healthy donors.

Because it has been shown by Miledi and colleagues that microtransplantation of native human neurotransmitter receptors in frog oocytes is a very powerful and relatively simple technique to perform [15,16], it is surprising that it has not been widely applied by other investigators. In this short review, we describe how we used this method to answer key questions that are difficult to address otherwise in the process of drug discovery [29,30]. The discovery of LY3130481 (also known as CERC-611), an AMPA receptor antagonist in development for the treatment of epilepsy, is used as an example to show how the technique has been used to demonstrate that the compound acts on native human AMPA receptors in brain regions associated with epilepsy but not in brain regions mostly associated with side-effects [30]. In addition, by microtransplanting bioptic hippocampal tissue obtained from an epileptic patient undergoing surgery, whether or not the compound acts on native AMPA receptors in the diseased brain is an issue that could be directly addressed [30].

Short history of the use of *Xenopus* oocytes for ion channel research and drug discovery

Oocytes from *X. laevis* are widely used as a convenient heterologous expression system to study the functional and pharmacological properties of various types of membrane proteins, such as ion channels, transporters and G-protein-coupled receptors (GPCRs). The use of oocytes as an expression system to study the function of ion channels was first described by Barnard *et al.* [31] over 35 years ago. They discovered that injection of mRNA isolated from the electric organ of *Torpedo* into *Xenopus* oocytes led to the expression of nicotinic acetylcholine receptors (nAChRs) in the plasma membrane of the oocyte and that the oocyte could subsequently be used to investigate the function of *Torpedo* nAChRs. Later, this technique was used to express mRNAs isolated from many different brain areas of different species and to study the function of various ion channels as well as crosstalk between GPCRs and ion channels [32].

With the emergence of molecular biology in the 1980s, and the cloning of huge numbers of different ion channels and receptors, *Xenopus* oocytes as an expression system became even more useful for ion channel research, and the technique made its official entry into the field of drug discovery. Electrophysiology using oocytes expressing recombinant ion channels has since been useful to study the mechanisms of drug action. The use of subunit chimeras and site-directed mutagenesis allowed, in many cases, the localisation of ligand-binding sites on receptor proteins and, with the aid of computational techniques, such as structural modelling, rational drug design became possible. Oocyte electrophysiology also allowed assessments of drug selectivity on receptor subtypes [33], as well as drug selectivity for different species [34]. The study of splice variants, mutations and ion channel polymorphisms causing human diseases (channelopathies) also benefited significantly from studies in oocytes [35].

In short, *Xenopus* oocytes have proved their importance in ion channel research and drug discovery. However, there are limitations in using the oocytes to express only isolated brain mRNAs or recombinant ion channels and receptors. Expression of brain mRNAs in oocytes has the disadvantage that mRNA from a mixture of heterogeneous cell types is injected in the eggs, resulting in receptor assemblies that might not reflect what is present in individual native cells. By contrast, expression of recombinant ion channels, while being a very powerful approach for molecular pharmacology studies, cannot completely reflect the native stoichiometry, the role of accessory subunits and the role of scaffolding and signalling molecules in modulating ion channel properties. Drug discovery aims at developing drugs to treat diseases in humans or, in a minority of cases, companion animals. It is therefore important to demonstrate that novel compounds identified by screening on recombinant human ion channels are also active on their native human counterparts. Interestingly, *Xenopus* oocytes have been found to be useful to address this question.

Microtransplantation of receptors and channels from native tissues into *Xenopus* oocytes

The experimental approach of injecting cellular membranes isolated from the electric organ of *Torpedo* into *Xenopus* oocytes was first established by Marsal *et al.* [36]. Membranes were prepared from *Torpedo* electric organ and upon injection into *Xenopus* oocytes these membranes were fused with the plasma membranes of the oocytes. In this way the oocytes acquired native nAChRs, which were already synthesised and fully assembled in the electric organ of *Torpedo*. The oocytes could subsequently be used to evaluate the physiological and pharmacological properties of native *Torpedo* nAChRs [37–40] using the standard two-electrode voltage-clamp technique [41].

A step forwards in the development of the microtransplantation technique was the demonstration that recombinant ion channels that were heterologously expressed in human embryonic kidney (HEK293) cells could also be microtransplanted into *Xenopus* oocytes by injecting membranes prepared from these transfected cells. This was shown for the following ion channels: human α4β2 [42,43] and α7 [42] neuronal nAChRs; rat iGluR1 ionotropic glutamate receptors [42]; and NR1/2b *N*-methyl-D-aspartate receptor (NMDA) receptors [44]. The functional and pharmacological properties of the ion channels that were microtransplanted in the oocytes were similar to the properties of the channels when they were evaluated directly by patch-clamping the transfected HEK293 cells.

Microtransplantation into oocytes of membranes from ion-channel-expressing HEK293 cells seems like a redundant step. However, it has been useful in helping to answer specific questions. For example, α4 and β2 nAChR subunits can assemble in two different subunit stoichiometries $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$. $(\alpha4)_2(\beta2)_3$ nAChRs have a higher sensitivity for the endogenous neurotransmitter acetylcholine than $(\alpha4)_3(\beta2)_2$ nAChRs. Because of the mixed stoichiometry obtained by expressing recombinant subunits, the concentration–response curve for ACh in cells expressing α4β2 nAChRs is usually biphasic in shape. nAChRs owe their name to the fact that they are the target of the tobacco plant constituent nicotine. Apart from activating or blocking

surface nAChRs, nicotine, being a membrane-permeable compound, also acts as a so-called 'pharmacological chaperone' [45,46]. By binding to premature, partially assembled nAChRs in the endoplasmic reticulum, nicotine influences subunit assembly and stoichiometry of the finally formed mature pentameric nAChRs [47]. Long-term treatment with nicotine causes an enhancement of the $(\alpha4)_2(\beta2)_3$ high sensitivity nAChR component in oocytes that was transfected with α4 and β2 nAChR subunit cDNAs. The same treatment had no effect in oocytes that were microtransplanted with membranes containing α4β2 nAChRs that were pre-assembled in HEK293 cells [43]. This result demonstrated nicely that the microtransplantation technique enables the study of receptors that are fully processed and assembled in the stoichiometry found in the original tissue.

Another step forward in the use of microtransplantation into oocytes of native membranes was the demonstration that it also works for studying native rodent γ-aminobutyric acid $(GABA)_A$ [48,49] and AMPA [29,50,51] receptors. In addition, nAChRs from innervated and denervated rat skeletal muscles were microtransplanted into oocytes and subtle differences in functional properties between nAChRs of the two tissues were demonstrated [52]. Results from another study showed that extracellular zinc in the central nervous system reduces pain via the NR2A subunit of NMDA receptors [53]. NMDA receptors containing the NR2A subunit with the single point-mutation NR2A(H128S) are not modulated by zinc, and NR2A(H128S) knock-in mice developed pain symptoms in various animal models for inflammatory and neuropathic pain. The microtransplantation method was used in this study to demonstrate that nanomolar concentrations of zinc inhibit NMDA receptors from wild-type mouse brains but not those in brains from NR2A(H128S) knock-in mice.

Microtransplantation of native human brain receptors into *Xenopus* oocytes

As described above, the microtransplantation technique was shown to be useful to answer basic questions about assembly and function of recombinant receptors expressed in HEK293 cells as well as native neurotransmitter receptors expressed in the fish electric organ and in rodent brain. Most exciting, however, was the demonstration that the microtransplantation technique could be applied to human tissues [15]. Even tissues that had been stored for >10 years in freezers from brain banks could be used and neurotransmitter receptors were found to be still functional once microtransplanted into *Xenopus* oocytes. Miledi *et al.* [15] used membranes prepared from the temporal neocortex of a patient who underwent surgery for intractable epilepsy and injected those into oocytes. Within a few hours the oocyte had acquired functional neurotransmitter receptors responding to GABA, AMPA, kainate and glycine. This result opened the way to detailed pathophysiological investigations into the role of ion channels in human brain disorders (Table 1).

Epilepsy

The microtransplantation technique has been applied most extensively in epilepsy research. The reason is that it is relatively easy to obtain tissue samples from epileptic patients undergoing surgical resection for refractory epilepsy. It is known that the GABAergic system is impaired in several forms of epilepsy [54–56]. In oocytes microtransplanted with membranes isolated from hippocampal and cortical tissue samples from epileptic patients, GABAergic impairment is manifested by a strong decrease of GABA responses upon repetitive stimulation with GABA [4,17,20–22]. The same phenomenon was observed when membranes prepared from hippocampal or cortical brain areas from experimental rodent epilepsy models were used for microtransplantation [57,58]. $GABA_A$ receptor rundown is believed to be a key property of drug-resistant epilepsy [20,22,57] and rundown of $GABA_A$ receptors can be prevented by phosphorylation of $GABA_A$ receptors [4,18,19,22]. Recent studies have shown that two clinically used antiepileptic drugs: levetiracetam [58,59] and lacosamide [23], restore this GABAergic deficit in epileptic patients and rodent models by reducing rundown of GABA responses. The most efficacious clinically applied antiepileptic drug combination is lacosamide plus levetiracetam. Lacosamide at concentrations that are not effective when applied alone is synergistic with very low doses of levetiracetam on GABAergic function [23]. The mechanism by which levetiracetam acts at $GABA_A$ receptors to reduce rundown is currently not known, but the restoration of $GABA_A$ receptor function by lacosamide is blocked by protein kinase C inhibitors [23], demonstrating that the effect of lacosamide is due to an interaction with a phosphorylation pathway of $GABA_A$ receptors and suggests that the drug does not exert its effect by directly binding to $GABA_A$ receptors.

Alzheimer's disease

Miledi *et al.* [24] further showed that membranes prepared from frozen post-mortem brains of humans who suffered from severe AD could be microtransplanted into *Xenopus* oocytes. AD cortical-membrane-injected oocytes responded to GABA with consistently smaller amplitudes than oocytes injected with cortical membranes from control non-AD brains [24,60]. The GABA-induced currents in oocytes injected with cortical AD and non-AD control membranes became larger during repetitive GABA applications [24]. This phenomenon clearly differs from oocytes injected with cortical membranes from human epileptic patients, which resulted in a marked run-down of GABA responses upon repeated GABA application [4,17,20–22]. In a subsequent paper, changes in $GABA_A$ receptor function in AD brains were examined in more detail [60]. $GABA_A$ receptors from AD brains were less sensitive to GABA than receptors from non-AD brains. GABA currents from AD brains desensitised faster than those from non-AD brains. Cortical receptors for glutamate have also been examined in oocytes injected with membranes from AD and non-AD brains [25]. Apart from being smaller in amplitude, the properties of cortical glutamate receptors from AD brains were essentially similar to those from non-AD brains.

Application of the microtransplantation technique to drug discovery

Perampanel

We have used the microtransplantation technique to address fundamental questions for our AMPA receptor antagonist project, which aimed at finding new ligands for the treatment of epilepsy [14]. First, we examined perampanel (Fycompa®), which is an established AMPA receptor antagonist drug used as an adjunctive treatment of partial-onset seizures [61]. Its antiepileptic activity is

TABLE 1

Summary of the use of human brain tissues to examine the physiological and pharmacological properties of neurotransmitter receptors microtransplanted into *Xenopus laevis* oocytes

Refs	Main result	Brain area	Receptors
[15]	First demonstration that injection of human brain membranes results in the appearance of functional human neurotransmitter receptors in the plasma membrane of *Xenopus* oocytes	Cortex	$GABA_A$ AMPA Kainate Glycine
[24]	Demonstration that microtransplantation of native human receptors from **Alzheimer's** patients into oocytes are functional and susceptible to detailed electrophysiological characterisation even after long post-mortem intervals	Cortex	$GABA_A$ Kainate AMPA
[18]	The rundown of $GABA_A$ receptors in oocytes microtransplanted with membranes from human **epileptic** tissue is counteracted by phosphatase inhibitors	Hippocampus	$GABA_A$
[19]	$GABA_A$ receptor function from the human epileptic hippocampus is different from those from control human hippocampus	Hippocampus	$GABA_A$
[81]	Rundown of **epileptic** $GABA_A$ receptors is decreased by the neurotrophin BDNF	Cortex Hippocampus	$GABA_A$
[20]	Rundown of $GABA_A$ receptor function in human **epileptic** patients	Cortex	$GABA_A$
[21]	Marked increase in rundown of $GABA_A$ receptor function in oocytes injected with membranes from **epileptic** patients than in oocytes injected with membranes from control individuals	Cortex Hippocampus	$GABA_A$
[77]	No difference found in functional properties of $GABA_A$ in oocytes injected with control hypothalamic membranes and similar membranes from a patient with **hypothalamic hamartoma**	Hypothalamus	$GABA_A$
[59]	The antiepileptic drug levetiracetam counteracts the $GABA_A$ receptor run-down observed in oocytes injected with **epileptic** cortical membranes; but not in oocytes injected with epileptic hippocampal membranes	Cortex	$GABA_A$
[25]	Responses to glutamate in oocytes injected with membranes from **Alzheimer's** patients are smaller than those of non-AD brains. The functional properties of glutamate receptors in oocytes injected with membranes from AD and non-AD brains were essentially the same	Cortex	GABAA Kainate AMPA
[78]	GABAA receptor rundown in **epileptic** patients is modulated by adenosine receptors	Cortex	$GABA_A$
[26]	Demonstration that microtransplantations of native human receptors from **autistic** brain into oocytes are functional and susceptible to detailed electrophysiological characterisation even after long post-mortem intervals	Cortex Cerebellum	$GABA_A$ AMPA Kainate
[79]	The rundown of $GABA_A$ receptors in oocytes microtransplanted with membranes from human **epileptic** tissue is counteracted by blocking adenosine A_{2A} and A_3 receptors	Cortex	$GABA_A$
[28]	Change in $GABA_A$ receptor pharmacology in human **Angelman syndrome**	Cortex	$GABA_A$
[22]	Rundown of $GABA_A$ receptor function in human **hypothalamic hamartomas**	Hypothalamus	$GABA_A$
[80]	Cortical $GABA_A$ receptors from **epileptic** patients have a more depolarised reversal potential (E_{GABA}) compared with $GABA_A$ receptors from nonepileptic controls. This difference of E_{GABA} was abolished by blocking NKCCI or unblocking KCC2. Western blotting of the same tissue samples revealed corresponding differences in expression levels of both chloride transporters	Cortex	$GABA_A$
[60]	Loss of functional $GABA_A$ receptors in brains from **Alzheimer's** patients	Cortex	$GABA_A$
[4]	Reduction of rundown of $GABA_A$ receptor function of **epileptic** patients by fractalkine/CX3CLI	Hippocampus Cortex	$GABA_A$
[29]	The antiepileptic drug perampanel inhibits human hippocampal and cerebellar AMPA receptors and it inhibits hippocampal AMPA receptors of an **epileptic** patient	Hippocampus Cerebellum	AMPA
[30]	The antiepileptic drug LY3130481 inhibits hippocampal AMPA receptors from **epileptic** patients; but not cerebellar AMPA receptors. This is consistent with the expression of the AMPA-receptor-associated protein TARP g-8	Hippocampus Cerebellum	AMPA
[44]	Evaluation of the use of synaptosomes rather than crude membranes for microtransplantation to specifically study synaptic neurotransmitter receptors	Cortex Hippocampus Cerebellum	$GABA_A$ AMPA NMDA
[23]	The antiepileptic drug lacosamide targets $GABA_A$ receptors and acts synergistically with levetiracetam, another antiepileptic drug. These drugs reduced the $GABA_A$ receptor rundown observed in oocytes microtransplanted with membranes from **epileptic** patients	Cortex	$GABA_A$

a result of AMPA receptor inhibition in the cerebral cortex and hippocampus. However, as a nonselective AMPA receptor antagonist, perampanel also inhibits AMPA receptors in brain areas that are not involved in epilepsy, such as the cerebellum, where blockade of AMPA receptors in rodent and human brain results in adverse motor side-effects [62]. Doses of perampanel that have anticonvulsant effects are within the same range as those causing the side effects.

By applying the microtransplantation technique to hippocampal tissue that was removed from a patient undergoing surgical resection for refractory epilepsy, we found that perampanel indeed inhibits AMPA receptors in brain tissue affected by the disease (Fig. 1) [29]. However, using the microtransplantation technique, we also showed that perampanel inhibits native rat and human brain AMPA receptors but does not discriminate between the hippocampus and the cerebellum, with AMPA receptors from both areas being equally affected (Fig. 2) [29].

Development of LY3130481: a forebrain-selective AMPA receptor antagonist

One of our goals was to develop an AMPA receptor antagonist to be used as an antiepileptic drug that blocks AMPA receptors in affected brain regions (cerebral cortex, hippocampus, amygdala, thalamus) while sparing AMPA receptors in other unwanted brain regions (e.g., cerebellum). We focused on the fact that the structure and function of AMPA receptors not only depends on the four pore-forming subunits comprising the ion channel itself but that also numerous AMPA-receptor-associated proteins exist that assemble with the receptor subunits to form larger receptor complexes. The first AMPA-receptor-associated proteins discovered

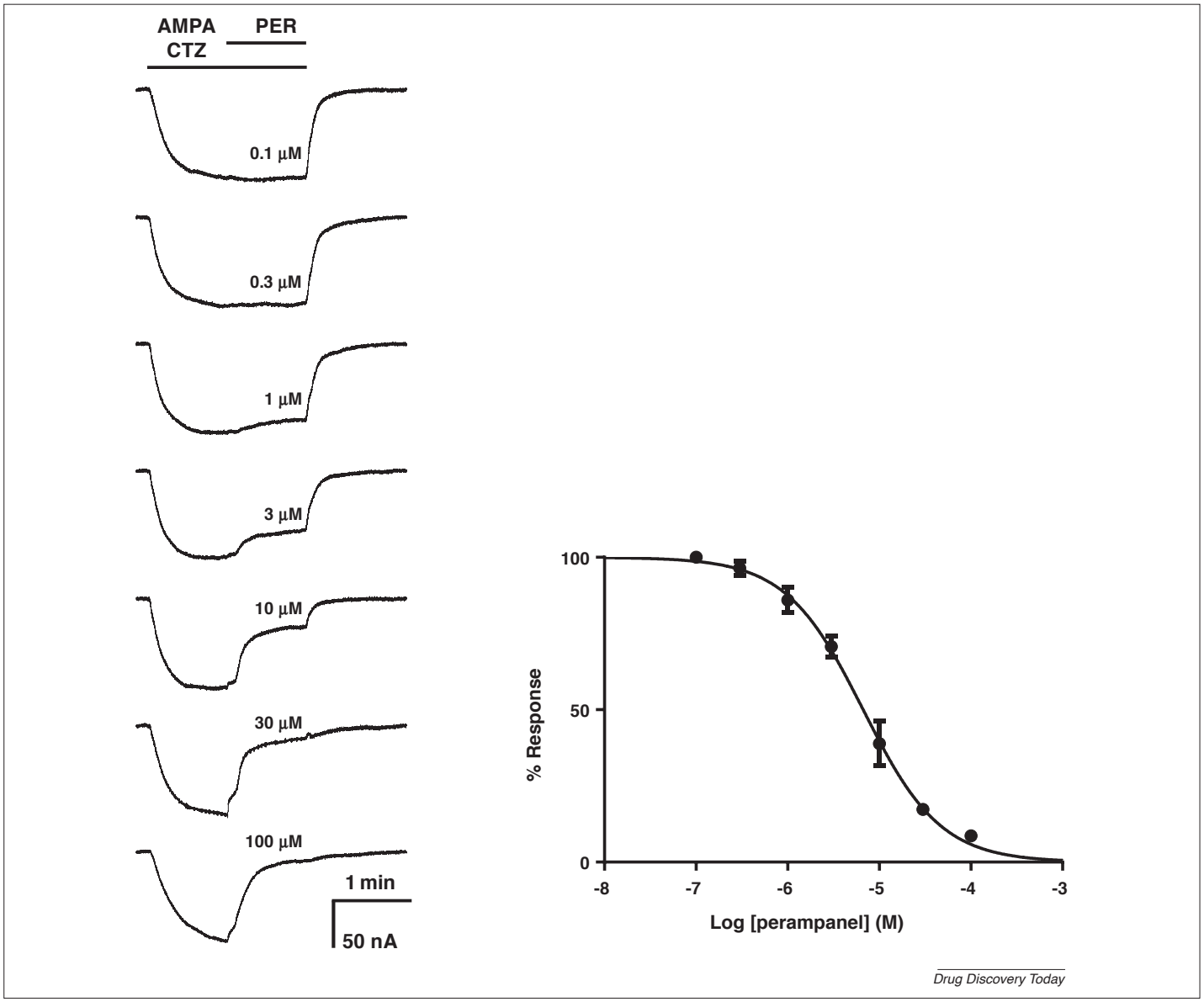

FIGURE 1

In oocytes microtransplanted with membranes prepared from hippocampal tissue from an epileptic patient, AMPA receptors were activated by application of AMPA/CTZ. Once responses to AMPA/CTZ reached their maximum, various concentrations of perampanel were applied. Perampanel inhibits AMPA/CTZ responses in a concentration-dependent manner and the concentration–inhibition curve is shown on the right, the EC_{50} value of perampanel to inhibit AMPA receptors is 6.7 μM. Reproduced, with permission, from [29].

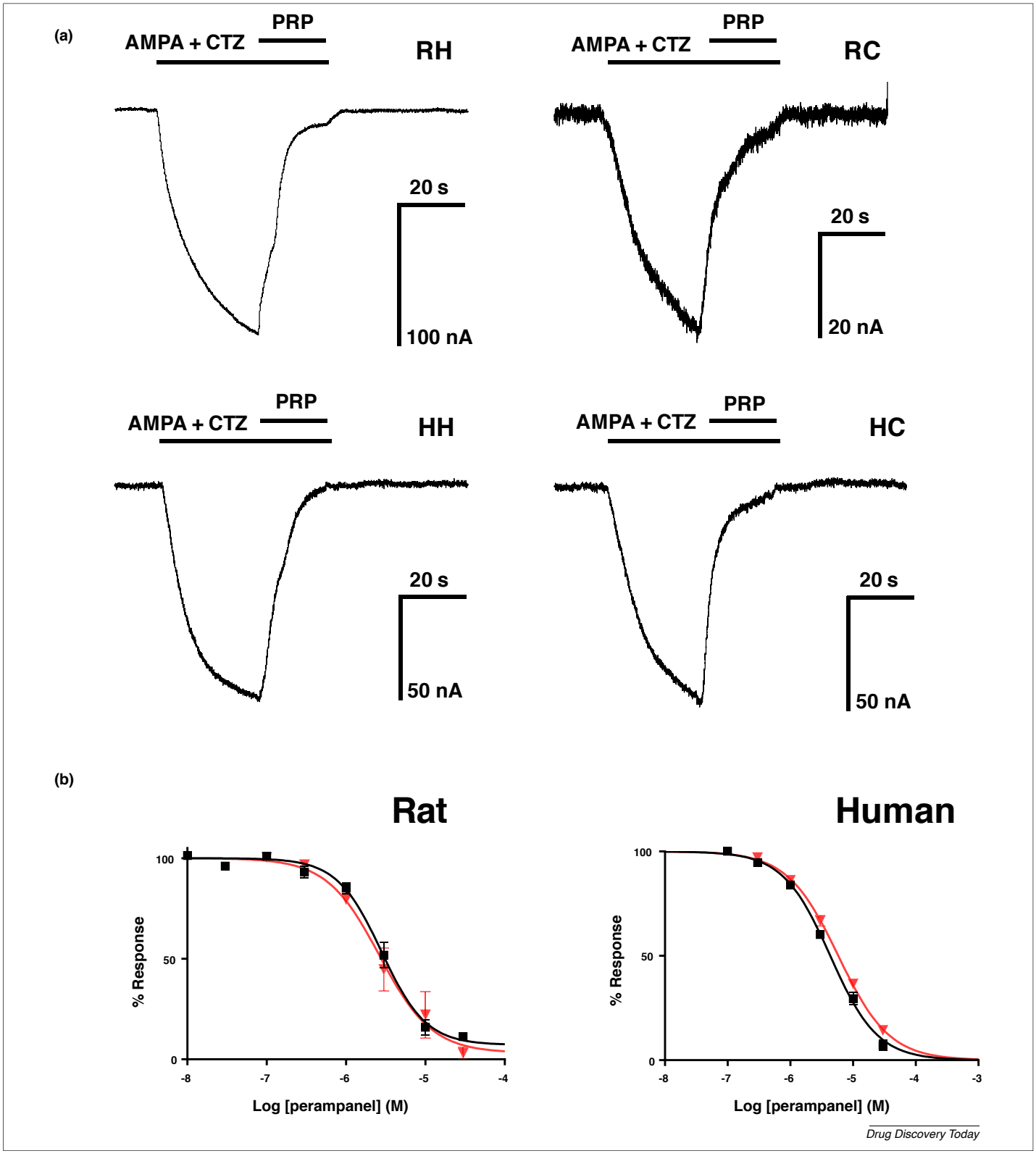

FIGURE 2

Membranes prepared from rat hippocampus (RH), rat cerebellum (RC), human hippocampus (HH) and human cerebellum (HC) were injected into *Xenopus* oocytes. **(a)** A high concentration of perampanel (30 μM) almost completely blocked the AMPA/CTZ responses in oocytes injected with each of the membrane preparations. The concentration–inhibition curves in **(b)** show that perampanel inhibited AMPA receptors in both brain areas and of both species with the same potency. EC_{50} values: RH = 2.8, RC = 2.6, HH = 4.3 and HC = 5.8 μM, respectively. Reproduced, with permission, from [29].

were the transmembrane AMPA receptor regulatory proteins (TARPs). TARPs are essential for neuronal AMPA receptor function in neurons – they enhance AMPA receptor trafficking to the plasma membrane and synapses, they modify channel gating [63,64] and they alter AMPA receptor pharmacology. For example, TARPs increase the potency of the positive allosteric modulator cyclothiazide [65], they convert certain competitive antagonists into partial agonists [66] and they increase the efficacy of the partial agonist kainate [67]. TARP γ-8 is selectively expressed in the forebrain where it modulates the pharmacological properties of AMPA receptors [64], but this protein is virtually absent in the cerebellum [68]. We built on this finding of a differential expression of the TARP proteins to discover LY3130481, a compound that selectively blocks AMPA receptors when assembled with TARP γ-8 but not with TARP γ-2 (which is highly expressed in the cerebellum) or other TARP isoforms [30,69]. As hypothesised, LY3130481 prevents multiple forms of seizures in rats and mice but did not produce the motor side-effects that were observed with nonselective AMPA receptor antagonists such as perampanel. As for perampanel, we have applied the microtransplantation technique to characterise LY3130481 selectivity (Fig. 3). LY3130481 blocks AMPA receptors in oocytes injected with human hippocampal membranes but not in oocytes injected with human cerebellar membranes. We showed that LY3130481 also acts on AMPA receptors from diseased brain tissue by testing the compound on oocytes that were injected with membranes prepared from hippocampal tissue obtained from a patient undergoing surgical resection for refractory epilepsy.

Concluding remarks and future perspectives

Xenopus oocytes are typically used for the expression of recombinant ion channels and receptors. In this short review, we have summarised how *Xenopus* oocytes have been used to assess the pharmacology and function of native receptors and channels obtained from rodent and human tissues. The advantage of the microtransplantation technique is that the receptors are fully assembled in the original native tissue. This is important because native receptors not only comprise pore-forming subunits but also accessory proteins that have formed complexes with them [67]. The finding that LY3130481 (a TARP γ-8 selective AMPA antagonist) blocks native human hippocampal AMPA receptors, but not the native cerebellar AMPA receptor, nicely confirms that native ion channels microtransplanted into *Xenopus* oocytes can remain associated with their accessory proteins. It also shows that accessory subunits are important for the function and pharmacology of the ion channel not only in recombinant settings but also in human brain tissues. In the context of drug discovery, this technique will be very valuable, because it helps answer important translational questions such as: is this drug candidate active on native human receptors in the right brain areas? Also: is a drug candidate active on native human receptors in the specific disease state?

Although the technique is simple and easy to establish, we found that it also has its limitations. It only seems to work for the main neurotransmitter receptors for inhibitory and excitatory neurotransmission. Microtransplantation works best for $GABA_A$ receptors. Responses are practically always seen upon application of GABA with every membrane preparation used. AMPA-type

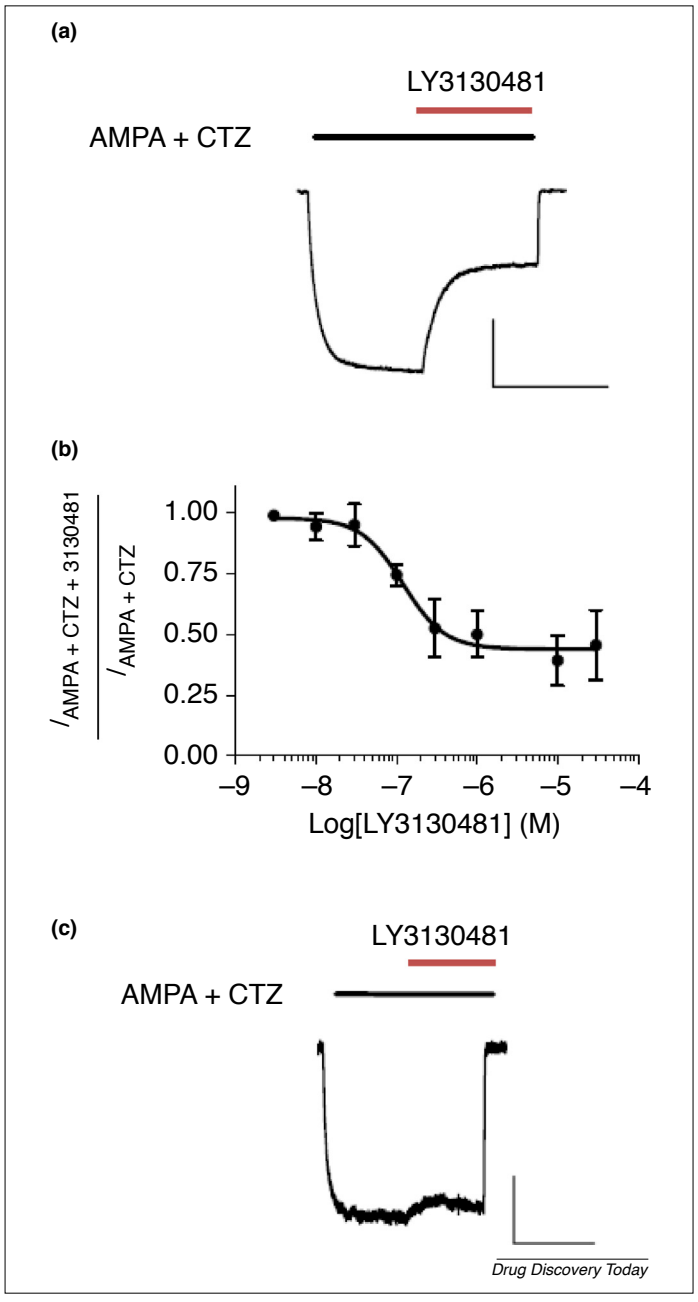

FIGURE 3

Effects of LY3130481 on epileptic human hippocampal AMPA receptors and human cerebellar AMPA receptors. **(a)** 10 μM LY3130481 partially inhibited AMPA/CTZ receptors from an epileptic patient. Scale bars represent 5 min/50 nA. **(b)** The IC_{50} of LY3130481 to inhibit epileptic human hippocampal AMPA receptors was 114 nM. Note that, unlike perampanel, LY3130481 did not produce full block of AMPA/CTZ responses. **(c)** Native human cerebellar AMPA receptors microtransplanted into *Xenopus* oocytes were not blocked by 30 μM LY3130481. Scale bars represent 5 min/20 nA. Reproduced, with permission, from [30].

glutamate receptors are also frequently detected, but responses to AMPA are very small and an AMPA receptor potentiator is usually used to be able to perform consistent experiments on AMPA receptors. In our hands, reasonably sized NMDA responses are occasionally seen, but their expression is inconsistent. There are no strong potentiators available for NMDA receptors like, for example, cyclothiazide for AMPA receptors. We and others have

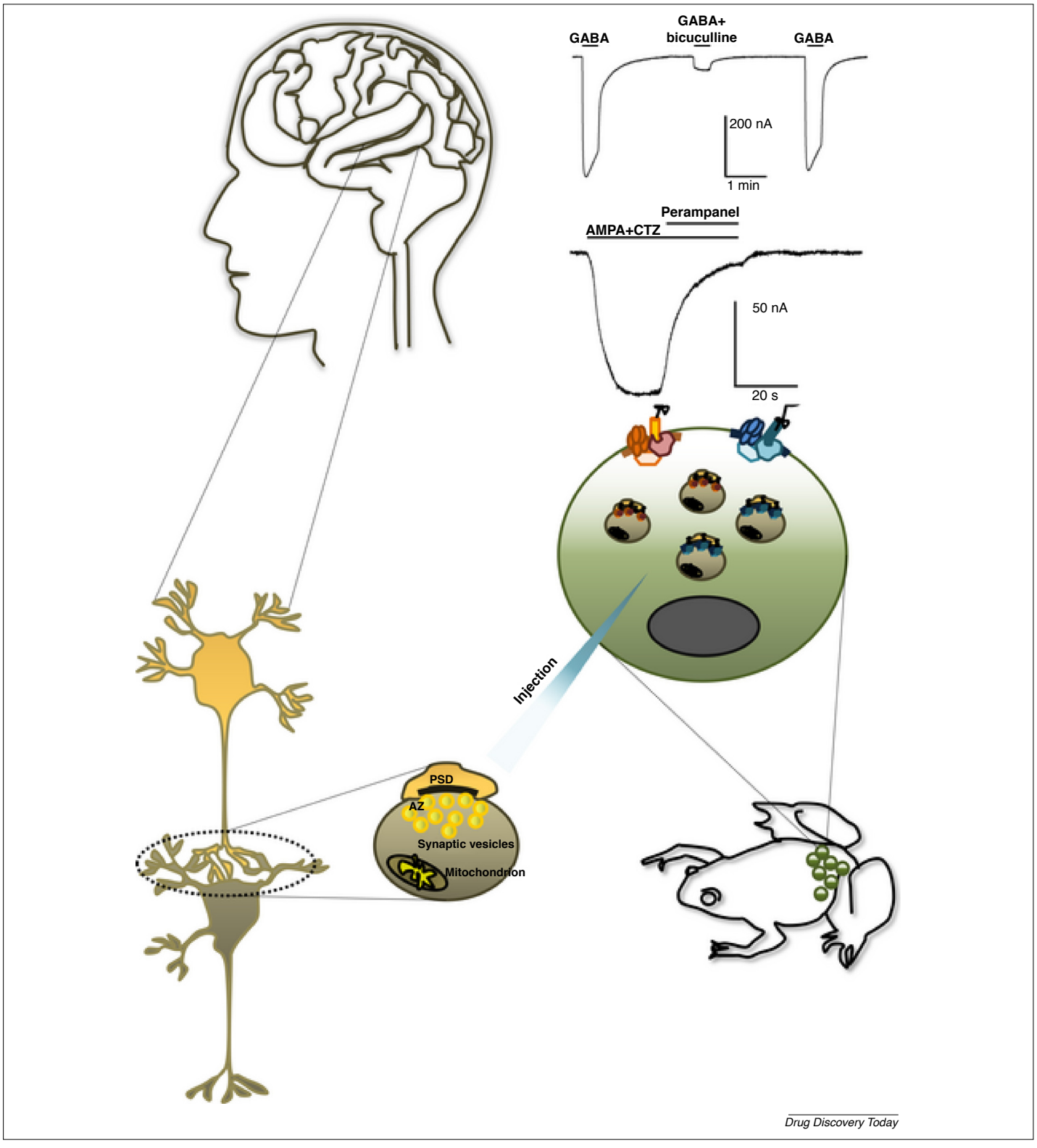

FIGURE 4

Cartoon showing the procedure to prepare synaptosomes from human brain tissue and the injections of synaptosomes into *Xenopus* oocytes. Frozen human brain tissues of brain areas of interest were obtained from brain banks. Upon homogenisation of the tissue, the tissue was centrifuged and synaptosomes were isolated using a sucrose gradient. The isolated synaptosomes were injected into *Xenopus* oocytes and after 1–2 days of incubation native human synaptic ion channels appeared in the oocyte's membrane. Ion currents through GABA and glutamate receptors were measured using the two-electrode voltage-clamp technique. The current traces on the top right show GABA- and AMPA/CTZ-mediated ion currents through native human $GABA_A$ and AMPA receptors microtransplanted into the oocytes. The GABA-induced currents were blocked by the $GABA_A$-receptor-selective antagonist bicuculline, and the AMPA/CTZ-induced current was blocked by the AMPA receptor antagonist perampanel. Reproduced, with permission, from [44].

also observed responses to glycine, but these have not yet been characterised in detail. There are no reports on microtransplantation of other neurotransmitter receptors, for example neuronal nicotinic acetylcholine, serotonin 5-HT3, purinergic P2X receptors. The reason is probably that their density in the brain is too low. Some evidence exists that the technique also works for voltage-gated ion channels, but this is not consistent and endogenous currents in the oocytes complicates the interpretation of the results.

Another weakness of the technique used so far is that a crude membrane preparation is usually used for microtransplantation. It would be very valuable if the technique could be used to more specifically study synaptic ion channels and receptors, because these are core to the emerging understanding of human synaptopathies [70–72]. In this vein, rat brain synaptosomes [73,74] and even post-synaptic densities [75] have been prepared and successfully used for microtransplantation. We have recently further evaluated the use of synaptosomes to study synaptic ion channels (Fig. 4) [44]. Synaptosomes were purified from different regions from rodent and human brain and these were first characterised biochemically for the expression of ion channel subunits and enrichment of synaptic proteins. In parallel, the same synaptosomes were mictrotransplanted in *Xenopus* oocytes. Also using synaptosomes, we found that LY3130481 inhibited rat hippocampal synaptic AMPA receptors but not rat cerebellar synaptic AMPA receptors, confirming that our previous findings on the brain-region-specific action of this drug candidate extends to synaptic AMPA receptors. Further, we detected similar changes in synaptic ion channel expression and function in synaptic membranes prepared from Alzheimer's brains were as previously found by others using conventional membrane preparations (Fig. 5) [25,44,60].

Although the technique of microtransplantation is relatively simple to establish, it has only been used by a handful of research groups. This is remarkable because the literature, and our own results, show that it is a very valuable technique for the elucidation of basic functional, pathological and pharmacological properties of native human ion channels and receptors. It is also very useful to address translational questions in drug discovery. The technique has great potential for future work. For example, we recently applied it to functionally validate synaptic proteomic findings that suggested heterogeneity in synaptic protein expression across different human cortical areas [76], which is a crucial early step before studying heterogeneity and channel modifications in pathological states.

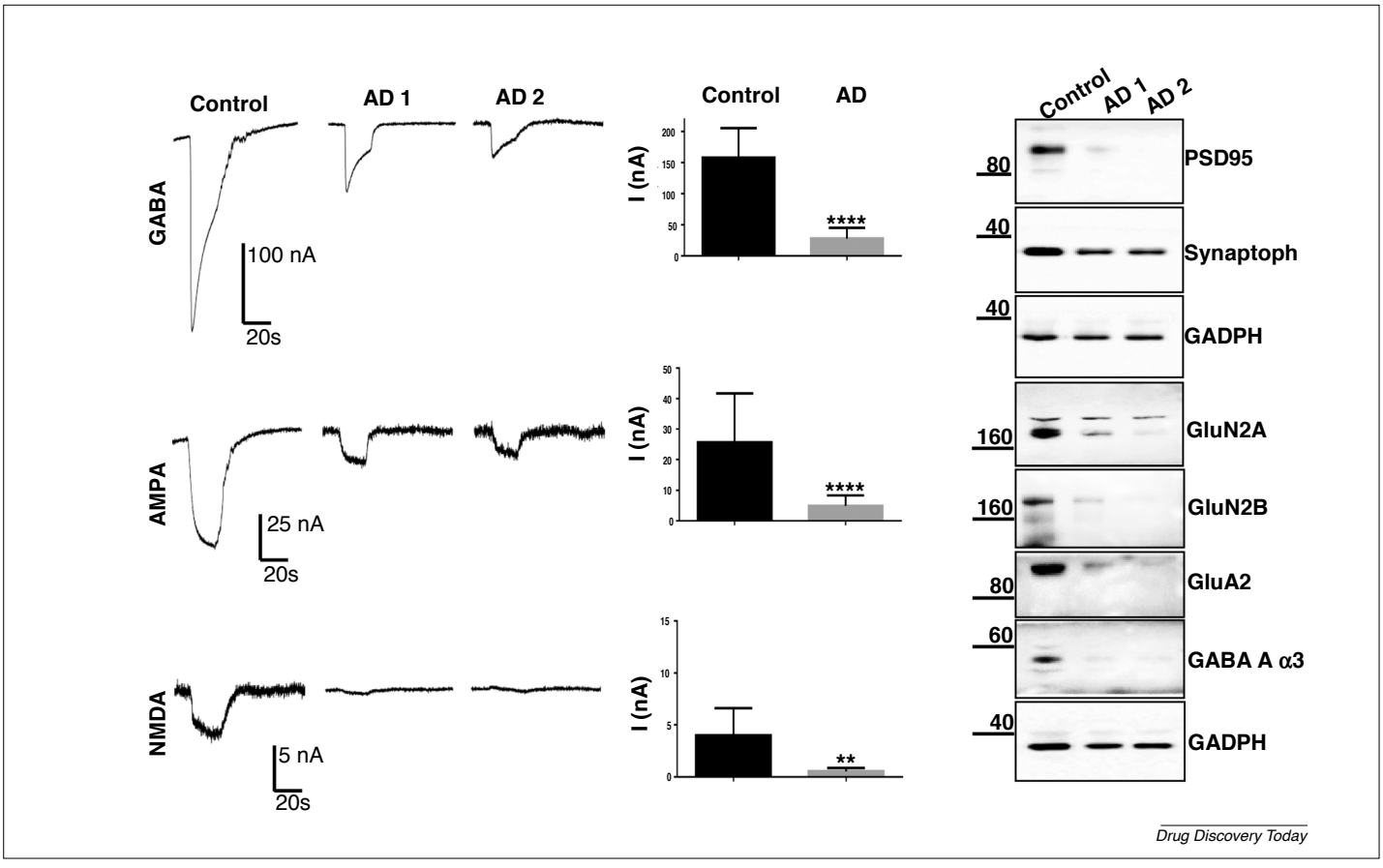

FIGURE 5

Synaptosomes prepared from post-mortem human cortex from a control donor and two patients suffering from Alzheimer's disease were injected into *Xenopus* oocytes. $GABA_A$, AMPA and NMDA receptor-mediated ion currents were recorded. Amplitudes of currents evoked by each of these neurotransmitters were much smaller in oocytes injected with membranes from the AD patients than in oocytes injected with membranes from the control donor. The same membrane preparations used for microtransplantation in oocytes were used in parallel for biochemical characterisation. Western blotting was used to evaluate the expression of synaptic markers and these experiments confirmed that membrane proteins for glutamate- and GABA-receptor subunits were much lower in membranes from the AD patients than those of the control donor. Reproduced, with permission, from [44].

Acknowledgements

This work was funded by Eli Lilly.

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