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Journal of Pharmacological and Toxicological Methods 51 (2005) 187–200

Journal of Pharmacological and Toxicological Methods

www.elsevier.com/locate/jpharmtox

Appraisal of state-of-the-art

HEK293 cell line: A vehicle for the expression of recombinant proteins

Philip Thomas, Trevor G. Smart*

Department of Pharmacology, University College London, Gower Street, London, WC1E 6BT, UK

Accepted 25 August 2004

Abstract

The HEK cell line has been extensively used as an expression tool for recombinant proteins since it was generated over 25 years ago. Although of epithelial origin, its biochemical machinery is capable of carrying out most of the post-translational folding and processing required to generate functional, mature protein from a wide spectrum of both mammalian and non-mammalian nucleic acids. Though popular as a transient expression system, this cell type has also seen wide use in stably transfected forms (i.e. transformed cells) to study a variety of cell-biological questions in neurobiology. The principal attributes which have made the HEK cell a popular choice among electrophysiologists to study isolated receptor channels include; its quick and easy reproduction and maintenance; amenability to transfection using a wide variety of methods; high efficiency of transfection and protein production; faithful translation and processing of proteins; and small cell size with minimal processes appropriate for voltage-clamp experimentation. These, and other attributes, also mean that complementary biochemical/cell biological evaluations of expressed proteins can be performed in concert with functional analyses to establish detailed pharmacological and biophysical profiles for the action of new drugs and their targets. The increased amount of sequence information available from the human genome has placed greater emphasis upon heterologous cell expression systems as targets for high throughput structure–function evaluation of novel drug targets and disease markers. Here we have highlighted some of the innate characteristics of the HEK cell in order that its suitability as a vehicle for the expression of a gene product can be assessed for particular needs. We have also detailed some of the standard methods used for transfection and obtaining functional data from electrophysiological recording techniques.

Keywords: Human embryonic kidney cell; Endogenous receptors; Transfection; Methods

1. History

The transformation of human embryonic kidney (HEK) cells following exposure to sheared fragments of human adenovirus type 5 (Ad5) DNA generated the widely used expression tool known today as the HEK293 cell line (hereafter referred to as the HEK cell). This permanently transformed cell line has incorporated Ad5 into chromosome 19 of the host genome, a modification used subsequently for the generation of recombinant E1-deleted (i.e. transcription incompetent) human adenoviral vectors. Until recently HEK cells were the only published Ad5-transformed human cell line, but subsequently human embryonic retinal (HER) cells have also readily been transformed with Ad5, and Ad12, though being of neuronal origin, this latter cell line is less suited to the study of neuronal proteins in isolation. The current commercially available source of HEK cell is from the original transformation by Graham, Smiley, Russell, and Nairn in 1977. The introduction of plasmid vectors such as those under the control of the CMV promoter (e.g. pcDNA, pCIS), besides many others, very effectively 'hijack' the HEK cell's synthetic protein machinery and force the translation of gene products artificially incorporated into the plasmid. Like the other transformed cell lines (Cos and CHO) discussed in subsequent chapters, the principle for the replication of the desired protein product is similar and will in many cases be achieved with the same plasmid

* Corresponding author. Tel.: +20 7679 3770; fax: +20 7679 7298.
E-mail address: t.smart@ucl.ac.uk (T.G. Smart).

1056-8719/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.vascn.2004.08.014

vector, it is only the cellular environment in which this is achieved which is different. However, these cellular vehicles are usually derived from different species and tissue origin and are therefore likely to vary in their efficiency of translation, export, and delivery to the target region and, most importantly, type and extent of post-translational processing (such as protein glycosylation and folding), or in the provision of appropriate intracellular signalling pathways.

The investigator must therefore be in a position to know whether the host cell provides other proteins which may be essential for function or stability. For example does the membrane protein being expressed require to be chaperoned to its functional locus; does it need to be anchored there and is there a requirement to link to intracellular signalling proteins (e.g. G-proteins); or downstream signalling elements (e.g. cAMP, kinases, phosphatases, calcium stores), to be functionally effective? Generally, the expression of neuronal membrane proteins such as Ligand-Gated Ion Channels (LGICs), Voltage-GICs, Acid Sensing Ion Channels (ASICs) and others, is faithfully reproduced compared to the native situation, in that the many signalling pathway elements common to native neurons are also represented in the intracellular milieu of the HEK cell.

For those reconstituted proteins which require accessory proteins in order to be functional, sometimes the HEK cell will provide (see Table 1), but if it does not then the cell can be co-transfected with the necessary gene product, or the cell can be simply transformed to stably express the necessary protein (see Table 2). An example of the latter would be the stably transformed HEK cell lines which over-express a variety of inwardly rectifying potassium channels, e.g. GIRK/Kir3 (Chuang, Yu, Jan, & Jan, 1998; Leaney, Milligan, & Tinker, 2000; Lei et al., 2000), which provide the platform for transient transfection with G-protein coupled receptors. As can be seen in Table 2, the number of stably transformed HEK cell lines which express specific proteins of use to the neuropharmacologist are numerous. However, the cell line is most useful as a transient transfection tool for the evaluation of pharmacological properties of multiple receptor subtypes reconstituted from individual subunit combinations, and for the rapid screening of multiple mutagenesis products for the study of structure–activity relationships of ion channel gating, signal transduction or receptor assembly characteristics.

Although the HEK transformed cell line may not provide the same sophisticated level of cellular architecture, subcellular organization or biochemistry associated with native neuronal preparations, it is invaluable as an accessible tool for the faithful reconstitution of many types of neuronal protein, obviating the topographical and biochemical complexities presented by the CNS. However, one recent and surprising caveat to this seems to be that Ad5 transformed kidney cells (as well as independently derived Ad12 transformed HEK cell lines), display some properties associated with a 'neuronal lineage' for these cells. This was initially revealed by the strong immunoreactivity HEK cells demonstrate for the four major neurofilament subunits normally expressed in neurones (Shaw, Morse, Ararat, & Graham, 2002). In addition to these neurofilament proteins, thus far a total of 61 mRNAs, which are normally specifically expressed only in neurones, can be detected in HEK293 (see Endogenous Receptors). As a consequence of these findings it is thought that the adenovirus specifically targets this neuronal lineage cell and in transforming it, turns on genes which have remained quiescent since the cell differentiated. Thus, this widely used human kidney cell line may have much more in common with early differentiating neurones than previously envisaged.

The reason for choosing one particular transformed cell line over another is manifold. This will include physio-

Table 1
Important neurophysiological receptors or protein subunits (detected as protein or mRNA) endogenous to the HEK293 cell

Ligand-gated	G-protein coupled	Voltage-gated and other[a]
Muscle acetylcholine (AChR) δ	Muscarinic AChR	Potassium channels: voltage-gated (Kv(α) 1.1, 1.2, 1.3, 1.4, 1.6, 3.1, 3.3, 3.4, 4.1, and Kvβ) and calcium-activated SK1
Nicotinic acetylcholine (nAChR): α7 and α5	Metabotropic (m) GluR: 1β and 4	Sodium channel: BNaC2 and β1A
Glutamate (GluR) 3	$GABA_B$ R1A	Voltage-gated calcium channel: $\alpha2_\beta$, $\alpha2_\delta$ isoforms I and β
γ-Aminobutyric acid ($GABA_A$ R): β3, γ3, and ε	5-Hydroxytryptamine: (5-HT) 1_D, 6, and 7_B	Protein tyrosine kinase (EPHB2)
Glycine (GlyR) β	Dopamine D2	PKA: catalytic and regulatory (R) II subunits
Acid Sensing Ion Channel (ASIC) 1a	Coticotrophin releasing factor 1 (CRF1)	PKC: α and δ
	Somatostatin type 2	Clathrin light chain α and β
	Bradykinin	Synaptotagmin
	Sphingosine-1-phosphate	Huntingtin interacting protein (HIP2)
	β_2 adrenoceptor	Trp1, 3, 4, 6 (mediators of store operated calcium entry)
	Purinergic (ATP/ADP) receptor $P2Y_{1\ and\ 2}$	Ryanodine receptor
	Adenosine A_{2B}	MKα (neurite outgrowth promoter)

R, receptor. This list is extensive but by no means exhaustive so it should not be assumed that because a protein is not mentioned it does not appear as part of the HEK cell's genetic makeup.

[a] Includes mRNAs usually exclusive to neuronal cells.

Table 2
HEK293 cell lines stably transformed with some examples of neuronal proteins

Receptor and subunit(s)	Cell line name	Reference
nAChR, α3β4	–	Stetzer et al., 1996
nAChR, α4β2	–	Gopalakrishnan et al., 1996
nAChR, α7	–	Gopalakrishnan et al., 1995
$5HT_{3A}$	–	Hope et al., 1996
NMDA R, NR1a/NR2B	EcR/rNR1a2B	Nagy et al., 2003
AMPA R, GluR4	–	Fletcher et al., 1995
Kainate R, GluR6 (EAA4)	–	Hoo et al., 1994
$GABA_AR$, α1β2γ2	–	Hamilton et al., 1993
$GABA_AR$, α1β2γ2	–	Besnard et al., 1997
$GABA_AR$, α3β2γ2	–	Besnard et al., 1997
$GABA_AR$, α5β2γ2	–	Besnard et al., 1997
$GABA_AR$, α1γ2	WSS-1[a] (WS-1)	Wong et al., 1992
$GABA_{C,}$ ρ1	–	Filipova et al., 1999
$P2X_7$	HEK-$P2X_7$	Gudipaty et al., 2003
Neuronal calcium channel (L-type), $\alpha1_D/\alpha_{2b}\delta$-1/$\beta_{3a}$	HEK 293 α1D	Bell et al., 2001
(N-type), $\alpha_{1B}/\beta_{1B}/\alpha_2\delta$	G1A1	Shekter et al., 1997
Ca^+-activated K^+ channel (I_K)	–	Jensen et al., 1998
$K_v1.5$	–	Wang et al., 2000
mAChR, M1 or M2 or M3 or M4	–	Peralta et al., 1988
GIRK1/GIRK2	–	Leaney et al., 2000
GIRK1/GIRK2/m4 AChR	G1,2m4	Chuang et al., 1998
GIRK1/GIRK4	G1,4	Lei et al., 2000
Kir6.2(and sulphonylurea SUR1)	–	Gopalakrishnan et al., 2000
AKAP75	HEK-A75	Feliciello et al., 1997
TrkB	HEK-TrkB	Narisawa-Saito et al., 2002
Adrenergic α_{2A} and α_{2C}	–	Bunemann et al., 2001

R, receptor; n/mACh, nicotinic/muscarinic acetylcholine; 5HT, 5-hydroxytryptamine; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid; Glu, glutamate; $GABA_{(A/C)}$, γ-amino-butyric acid (Type A or C); I_K, voltage-independent, inwardly rectifying K^+ channel; K_v, voltage-gated K^+ channel; GIRK, G-protein coupled inwardly rectifying K^+ channel, also known as Kir; P2X, purinergic (ATP) receptor; Trk, tyrosine kinase receptor; A-Kinase anchoring protein (AKAP).
[a] ATCC accession code: CRL-2029.

logical reasons such as the nature of the endogenous receptors, proteins, or signalling pathways, and also practical and cost considerations. For example, appropriate questions to ask before choosing a cell line might be: what is the expression efficiency of the cell; is the cell size appropriate for the experimental manipulation; is the cell division rate appropriate; is it amenable to downstream manipulations (e.g. biochemical or immunocytochemical procedures)? Many of these considerations will be outlined in the ensuing sections.

1.1. Endogenous receptors

The principal reason for using cell lines in preference to native neurones for the study of recombinant hetero-oligomeric ion channels of relatively defined subunit composition, is that their properties may be studied in isolation from other receptors of the same, or different, families. However, depending on the source of the parental cell line there may be interference from endogenous proteins constitutively expressed by the host cell. For HEK cells, potential complications to the study of ionic currents can arise, for example, due to the presence of endogenous voltage-gated potassium channels (I_K), of which there are many characterised (see Table 1). Although the majority of recombinant expression studies using electrophysiology involve some sort of voltage clamp protocol, the presence of these channels is still significant (especially when expression of the exogenous protein is low), as they generate currents in the range of several hundred picoamps upon depolarisation, and display conductances and current densities comparable to native I_K in hippocampal and cortical neurons (Yu & Kerchner, 1998). The range of proteins constitutively present in the HEK cell is not limited to voltage-activated channels such as calcium channels (Berjukow et al., 1996) or the acid-sensing ion channel, ASIC1a (Gunthorpe, Smith, Davis, & Randall, 2001), as many LGICs, G-protein coupled receptors and intracellular regulatory proteins of neuronal origin have also been found (Table 1). Many of these have been identified by electrophysiology or biochemical assays and subsequent RT-PCR methods have extended the potential number of contaminating or accessory proteins present in the HEK cell. Presently, there are a surprisingly large number of proteins, or at least mRNAs, of neuronal origin which can be detected to varying extents in the native HEK cell. It is therefore imperative that HEK cells of unknown origin are assessed prior to transfection for their functional profile, as alterations in batch-to-batch phenotype evidently occurs (see also Section 4.2).

An extensive study by Shaw et al. (2002) using microarray detection strongly suggests the presence of mRNA for 28 G-protein coupled receptors—for example mGluR4, $GABA_B$-R1a, and $5\text{-}HT_{7b}$ (Table 1), implying that G-protein coupled signalling pathways involving diacyglycerol (DAG), IP_3, and calcium are operational in the HEK cell. Among the mRNAs uncovered for ligand-gated channels were significant levels of acetylcholine receptor α5, α7, δ, and Glycine receptor β and $GABA_A$ receptor γ3 and ϵ subunits in addition to the glutamate receptor subunit GluR3 (Table 1). Some of these mRNAs and their protein products, which could profoundly influence the phenotype of the transiently expressing cell, have been detected by independent studies. Notably, Dautzenberg, Higelin, and Teichert (2000) have reported endogenous corticotrophin releasing factor type I (CRF1) receptors (also present in COS-7 cells) which could potently increase intracellular levels of cAMP. Some of these endogenous proteins and channels far from providing, for example, the correct substrates for signalling cascades, can interfere with exogenous protein function. For example, the HEK cell may be unsuitable as a vehicle to study P2X receptor-

mediated calcium influx due to the existence of endogenously expressed calcium-mobilizing purinergic P2Y receptors which would effectively be co-activated by ATP (He, Zemkova, Koshimizu, Tomic, & Stojilkovic, 2003). Similarly, the endogenous splice variant of the β1 sodium channel subunit (β1A), a channel responsible for the initiation of the action potential in excitable cells, is a subunit which co-assembles with the equivalent α subunit (Nav1.4) when expressed in HEK cells, thereby reducing the slow mode of channel inactivation (Moran, Nizzari, & Conti, 2000). However, although RT-PCR evidence for β1A in HEK cells is irrefutable, subsequent experiments have re-assigned its role from that of functional modulator to that of establishing contact with cytoskeletal or extracellular matrix proteins (Moran, Conti, & Tammaro, 2003).

Of particular relevance to the GABA receptor field of study is the observation that non-transfected WSS-1 cells (HEK cells transformed to stably express α1 and γ2 $GABA_A$ receptor subunits), have revealed the presence (using RT-PCR) of an endogenous β3 $GABA_A$ receptor subunit, whose GABA-activated current is sensitive to bicuculline and the subunit selective agents diazepam and loreclezole (Davies, Hoffmann, Carlisle, Tyndale, & Hales, 2000). Indeed in α1 and γ2 transiently transfected HEK cells, which are normally insensitive to agonists (Connolly, Krishek, McDonald, Smart, & Moss, 1996; Sigel, Baur, Trube, Mohler, Malherbe, 1990), the endogenous β3 subunit is occasionally able to chaperone these subunits to the cell membrane and form functional receptors. Though difficult to replicate, similar observations have been made by Ueno, Zorumski, Bracamontes, & Steinbach (1996) who, as well as observing the presence of the endogenous loreclezole sensitive β subunit under conditions of low expression, were also able to elicit responses to GABA on some non-transfected HEK cells. The passage number of the cells, culture media conditions, or original cell source has been speculated to be a potential source of discrepancy between studies which report endogenous GABA-activated currents in HEK cells (e.g. Fuchs, Zezula, Slany, & Sieghart, 1995) and the large majority of those which do not. These observations highlight the variability in expression levels of endogenous proteins, as the β3 subunit was not picked up in the mRNA screen of principal neuronal proteins by Shaw et al. (2002), and several laboratories have been unable to detect the expression of endogenous β3 subunits (Taylor et al., 1999; Wooltorton, Moss, & Smart, 1997). Our laboratory has consistently failed to detect GABA-activated currents from non-transfected HEK cells, or the presence of β3 mRNA using RT-PCR.

Although present to some degree in the parental HEK cell line, endogenous neuronal subunits are most probably out-competed during assembly of the final receptor complex following the high expression levels achieved with the exogenously introduced subunits, and are therefore considered to have minimal contaminating influence on the expressed receptor populations. The use of powerful promoters in established vectors aids this process. For example, using the Superfect (Qiagen) transfection reagent, Dautzenberg et al. (2000) report expression rates of recombinant receptors (CRF1) of up to 400-fold higher than those calculated for the same endogenous protein in HEK cells, though this figure will obviously be dependent on the role of the endogenous protein and its distribution within the cell. The observations outlined in this section none-the-less serve notice to be aware of these potential physiological or biochemical contaminants when choosing the cell system in which to reconstitute a particular neuronal protein, and to perform regular analyses of control HEK cells, especially when obtained from sources of unknown origin.

2. Methods for preparation and maintenance of HEK cells

HEK cells can be sourced from American Type Culture Collection (ATCC: Manassas, USA) under the designation '293', accession code CRL-1573. As well as a repository for the original Ad5 transformed cell line, ATCC also maintain another variant called HEK 293E, which has been transformed by the Epstein–Barr (EBNA1) virus. This, along with an SV40 virus transformation product called HEK 293T, is not as widely used in expression studies as '293'. Purified genomic DNA from the '293' cell line is also available from ATCC should the extent of potential protein contaminants need to be established. These cells do not adhere to substrate unless kept at 37 °C so they can be transported without mechanical damage in suspension. Generally, cells can be continuously cultured at 37 °C in a 5% CO_2:95% air humidified incubator, though their health (i.e. morphology, growth rate, translational efficiency) will deteriorate as the passage number increases. As a general rule, a new stock of cells acquired from ATCC should initially be grown until they are ~70% confluent, then passaged (i.e. diluting their density by lifting and replating) on two consecutive occasions and then stored frozen under liquid nitrogen in quantities which are sufficient for a number of years. This stock of HEK is regarded as 'laboratory passage one'. Cells defrosted and plated from this stock are generally reliable in terms of their native phenotype, transfection efficiency and electrophysiological stability for 20–30 passages before a new stock needs to be thawed and regenerated. HEK cells remain biochemically active at 50–70% confluency, and are enzymatically harvested when reaching 70% confluence for immediate transfection by 'electroporation' or 'nucleofection' (see below). Cells at this stage can also be seeded at 30–50% density if the transfection procedure involves a DNA precipitation reaction as used in the 'calcium phosphate' method; the formation of micelle structures (e.g. Effectene,

Qiagen); or a liposome-based incorporation step (e.g. Lipofectamine 2000, Invitrogen).

Cells which have been allowed to go through multiple cell divisions, which happens over a period of 36–48 h following transfection, will form micro-islands with individual cells electrically coupled to each other by gap junctions (see Fig. 1). Though not as ideal as recording currents from single cells, the gap junction is beneficial if only one cell of a 2–3 cell cluster is transfected, but is generally detrimental to successful voltage clamping techniques on larger clusters due to the increased area/volume and associated space clamp errors. Therefore, it is imperative that cells are initially plated as single entities following transfection.

2.1. Preparing cultures of HEK cells

HEK cells growing on 10 cm culture dishes are harvested with 5 ml of trypsin–EDTA (0.25% (w/v) (Gibco) applied for a 2 min incubation) following removal of the culture medium (DMEM, see recipe below) and an HBSS (Ca^{2+}/Mg^{2+} free) wash. Cells are seen to lift from the dish at which point culture medium (10 ml) is added to quench trypsin activity and the harvested cells are sedimented (85×*g*, 5 min). At this point, if cells are going to be used for electroporation, then refer to the relevant section below. If, however, cells are to be replated either for stocks or calcium phosphate/micelle-based transfection (see Section 2.2), then the pellet is resuspended in culture medium at this point. This resuspension volume is small (1 ml per 10 cm dish) so that the following trituration step with a fire polished (to half orifice width) Pasteur pipette, is effective in breaking down the pellet into individual cells. Cells are then either plated directly onto 10 cm dishes (plating stock) or onto pre-prepared (see below) poly-L-lysine coated coverslips (ideally 12–22 mm) if required for electrophysiology. The density for stock dishes must be sufficiently low to achieve 70% confluence 2–3 days later, and ideally should approximate to 5×10^3 cells per 22 mm coverslip for calcium phosphate/micelle-

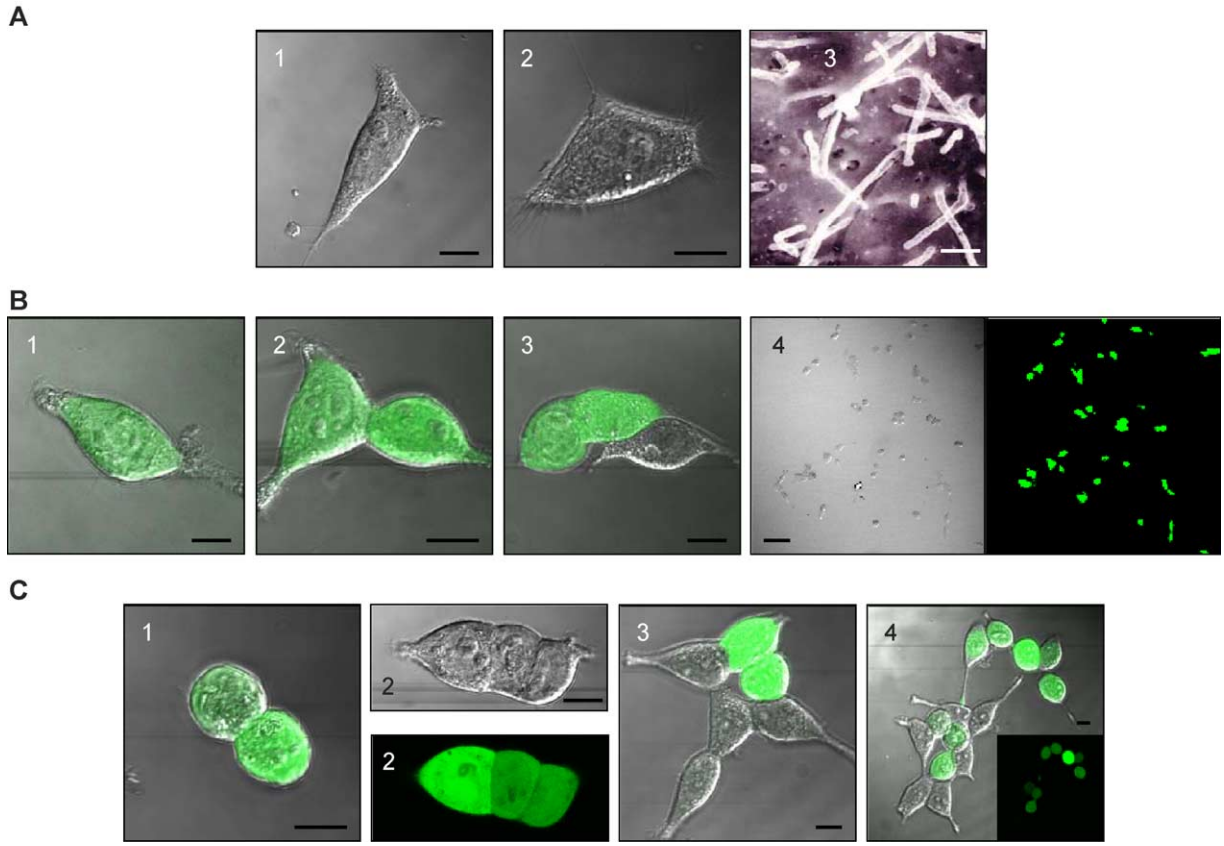

Fig. 1. HEK 293 cell morphology and suitability for whole-cell electrophysiological recording. A, Transmitted light images of single, non-transfected HEK cells demonstrating their classical pyramidal or rhombic morphology and associated processes (A1), and smaller hair-like filopodia around the membrane periphery (A2), which are more obvious under scanning EM visualisation (A3). B, Examples of reporter gene protein EGFP (green) transfected HEK cells which are viable for electrophysiological recording. Healthy cells maintain their morphology once transfected. The ideal situation for performing voltage/current clamp experiments is the single cell (B1), though electrically coupled pairs are also suitable (B2). More mature cultures (>2 DIV) will contain clusters of cells which are also viable, but if chosen should be limited to three cells, not all of which should be transfected (B3) in order to limit the size of the whole-cell currents. Panel B4 demonstrates the efficiency of the calcium phosphate transfection technique, in this case achieving greater than 90% efficiency. C, HEK cells unsuitable for electrophysiological recording are those that either look unhealthy (i.e. rounded-up, C1), will produce too large a current due to over-transfection (i.e. all cells in a small cluster are transfected, C2), or which are associated with larger cell clusters (C3, C4). All scale bars are 10 μm, except A3 (0.5 μm) and B4 (100 μm).

based transfection the following day. At this point with either the cells plated or in suspension you are in a position to choose whichever transfection procedure best suits your requirements.

2.1.1. Reagents and coverslip preparation

DMEM-based culture media contains DMEM+sodium pyruvate: 10% (v/v) foetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin and 2 mM L-glutamine. For coverslip substrata coating, 0.5 ml of poly-L-lysine hydrobromide (0.1 mg/ml in sterile water) is placed onto 100% ethanol flame-cleaned coverslips (borosilicate, thickness No. 1.5, Cat. No. 406/0189/44; BDH) in 35 mm dishes or their equivalent. The solution is left to coat the glass for at least an hour after which time it is removed by suction and the coverslip is washed three times with sterile water before being left to dry for 2 h at room temperature.

2.2. Transfection techniques

The ability to generate transient HEK cell phenotypes by the introduction of exogenous nucleic acid depends on a range of transfection techniques. Transfection efficiencies using these techniques are variable and particularly sensitive to the quality of the reagents used in the preparation.

2.2.1. Electroporation

Cells are exposed to an intense electric field (field strength=1 kV/cm) causing transient perforations to form in the membrane through which the nucleic acid is purported to diffuse. We generally use cDNA as it is more stable than cRNA, which also has a finite lifetime in the cell. The transfection efficiency (i.e. the number of transfected cells as a proportion of the total number of cells) is routinely 30% but can be as high as 80%. Routine transfection rates of up to 80% have been reported for the related technique of Nucleofection (Amaxa/Qiagen), which attaches a cell specific nuclear localisation signal to the nucleic acid complex prior to transfection.

2.2.2. Nucleic acid preparation

Athough not absolutely necessary, we would advocate that any cDNA that is used for transfection of HEK cells is prepared in endotoxin-free buffers. In this technique, transfection of a 500 μl sample of cell suspension occurs in an electroporation cuvette (Biorad) and requires 10 μg of final DNA (added in a 10 μl volume). Approximately 25% (2.5 μg) is assigned for the reporter gene (e.g. Enhanced Green Fluorescent Protein, EGFP) to identify transfected cells. While subunit stoichiometry can vary between different receptors, if assembly is tightly regulated, it is only usually necessary to mix equal combinations of most receptor subunits and achieve near identical functional receptors compared to neuronal isoforms. Thus, the remaining 7.5 μg of DNA should be split equally between the subunits which will form the final protein complex (e.g. 2.5 μg of DNA for α1, β2, and γ2 subunits for the principal isoform of the $GABA_A$ receptor). The constituents must be well mixed without aeration.

The cell pellet from the sedimentation procedure (Section 2.1) is washed (several triturations with a wide bore pipette) in 25 ml (irrespective of the number of plates harvested) of Optimem (with glutamax-1) before re-sedimentation (85×*g*, 5 min). The supernatant is removed and 500 μl of Optimem is added for each transfection (i.e. each cuvette) to be performed. A further 500 μl of Optimem is also required to be added at this stage to re-plate cells from this procedure, in order that the laboratory stock is continued (e.g. a proportion of this 500 μl of cell suspension is seeded into 10 ml of DMEM-based culture medium in 10 cm dishes). Cells are resuspended by trituration (10 times) with the half-bore fire-polished Pasteur pipette. If one 70% confluent 10 cm dish of cells is used for each transfection this will provide the correct cell density (~30×10^3 cells) in the electroporation cuvette.

Cells and DNA are mixed in the cuvette (Biorad—0.4 cm electrode gap, sterile, Cat. No. 165-2088) by gentle finger stroking (causing minimal aeration). The Gene Pulser II (Biorad) settings we have consistently used for this cuvette are: infinite resistance (Pulse Controller), 125 μF capacitance, and 0.4 kV voltage. While the cells are still in suspension, the voltage is discharged, the cuvette removed, and the cells are re-mixed prior to one further discharge (this seems to increase efficiency significantly). Finally, re-suspend the cells in situ (finger stroke) after which the cells must be left for a 'relaxation' period of 10 min. If cells are further agitated at ~3 min intervals during this period the efficiency improves considerably.

Remove only live cells from the cuvette (i.e. those in suspension, not the dead cells which accumulate in the 'froth' at the surface), dilute and suspend in 10 ml of DMEM growth medium. Plate 150–300 μl of this suspension onto each coverslip (those to be used on later experimental days should be less dense), add a further 500 μl of culture medium (for 22 mm coverslips) and swirl gently in order to spread the cells over the coverslip only. Cell density should be checked under the microscope at this stage and can be adjusted accordingly before adherence. Leave cells to fully settle and adhere for 30 min at 37 °C before adding a further 1 ml of DMEM to each dish. We have recorded whole-cell nano-Amp currents from cells 3–4 h after transfection and for 3–4 days thereafter. Only a small fraction of the electroporated cells are plated for use in electrophysiological assays, thus there is ample scope to plate sufficient numbers of cells for biochemical assays.

2.2.3. Calcium phosphate precipitation

This method is a very inexpensive and accessible method of cell transfection as no specialist equipment is required. However, we have found that the efficiency can vary widely, usually due to the variable pH associated with media of different maturities, and the critical pH required for the

HBSS media (see below). It is also considerably less labour intensive than electroporation and the calcium–DNA precipitate does not have to be removed for continued cell survival. The actual cell uptake and nuclear transport of the cargo DNA is still not well understood, but the technique is amenable to large scale production of transfected cells so is suited to many cell biological procedures. The disadvantage associated with this method is that if the initial seeding density of cells is low then success rates can be unpredictable, and DNA precipitation must not be allowed to reach a level whereby large aggregates form. This is critically dependent on the pH of the solutions.

Prepare a mixture of 4 μl of DNA (2 μg total per 22 mm coverslip) with 20 μl of $CaCl_2$ for each transfection. Mix this with 24 μl (per coverslip) of double strength HBSS. Allow no more than 20 min for a DNA precipitate to form. If the mixture is not left for long enough then insufficient precipitate forms and transfection rates fall; if left too long then the precipitate can form large particle aggregates and either not be taken up by the cell or taken up in too large a quantity becoming cytotoxic. HEK cells plated on 22 mm coverslips as described in Section 2.1, are washed with HBSS (w/o Ca^{2+}/Mg^{2+}) and medium replaced with 1.5 ml of DMEM-based growth medium. 45 μl of the DNA:$CaCl_2$ mix is added drop-wise over the plated cells and the dishes are returned to the incubator (37°C, 5% CO_2:95% air) without any disturbance of the dishes. Media can be changed 12 h later if cytotoxicity is apparent.

2.2.3.1. Reagents. Filter sterilized double strength HBSS (280 mM NaCl, 2.8 mM Na_2HPO_4, 50 mM HEPES, exactly pH 7.2) and $CaCl_2$ (340 mM) are stored as aliquots and defrosted on ice as needed. DNA (2 μg in total for each coverslip) is divided as 0.5 μg for the reporter gene, and the rest equally divided among subunit nucleic acids.

An alternative but very similar method to that of calcium phosphate precipitation uses one of many similar commercial products called *Effectene*. We find this to be at least as successful as calcium phosphate and often more able to deliver larger amounts of DNA into the cell. It has the added advantage of not being compromised by the presence of serum or antibiotics in the medium, unlike other commercial products which could disadvantage transfection of clonal cell lines that need to be selected for growth in an antibiotic. The principal is also one in which DNA is condensed and then complexed to the Effectene reagent in the formation of consistently sized micelles, before being exposed to the cells. Following the manufacturers protocol, it is possible to achieve 10–30% transfection efficiency and persistence of the reagent in the medium around the cells is not generally cytotoxic, and generally improves efficiency. The technique is also effective on cells in suspension. The advantage the cell-attached (i.e. not in suspension) transfection methods afford is that it is possible to perform multiple transfections on consecutive occasions on the same cells.

2.2.4. Other techniques

Lipofectamine 2000 represents one of the more recent cationic lipid encapsulation reagents which deliver DNA to the cell by fusion and incorporation of lipid microsomes into the cell's lipid bilayer. Though fairly efficient, we have found that being cationic, a negative charge can build up in the membrane as a result, making patch-clamp manipulations very difficult during the initial 24 h. Whether this charge gradually dissipates in culture is enigmatic but the cells eventually become useable after 24 h. This may not be a problem for other cell types or particular types of receptor/protein. There are in excess of 16 different commercial sources of transfection reagents, many of which offer more than one method for plasmid delivery, such as via polycationic lipids, liposomes, dextrans, activated dendrimers, and even receptor mediated endocytosis. Biolistic approaches are not used as a matter of routine for cultured cells as these techniques are more suited to larger tissue masses where DNA penetration deep into a tissue slice may be required. Likewise, nuclear micro-injection is a high risk, labour intensive method, but as with biolistics, could be necessary for those nucleic acid cargoes which do not readily get taken up by the HEK cell.

3. HEK298 cells for electrophysiology

3.1. Choice of cell

With a resting membrane potential (R_p) of around −40 mV the HEK cell clearly displays electrotonic properties which are somewhat different from most neurons. We would routinely voltage clamp a HEK cell at or near its R_p (i.e. generally −20 to −40 mV). Given that the chloride concentrations in our intra- and extracellular solutions are balanced (i.e. 0 mV equilibrium potential) across the membrane in which our GABA-gated chloride channel is expressed, a holding potential of −40 mV provides more than sufficient driving force to consistently record maximal currents of up to 5 nA in the whole-cell voltage-clamp configuration. Thus the signal-to-noise ratio is high obviating the need for additional filters when recording whole-cell currents. As alluded to in Section 1, transfected cells which are most suitable for electrophysiological recording will be those which satisfy most of the following 10 characteristics, some of which are summarised in Table 3:

1. Demonstrate moderate reporter protein (GFP) fluorescence (highly expressing cells are usually unhealthy).
2. Are ideally single cells with no discernable connections to other cells. Cell couplets, or triplets at worst (only one of which need be transfected), are also viable, though membrane voltage-clamping becomes more problematical.

Table 3
Whole-cell biophysical properties of healthy, single HEK293 cells

R_p (mV)	Seal resistance	Holding current (at −40 V_H)	Capacitance[a] (pF)	Series resistance (MΩ)	Input R (MΩ)	I/V characteristics[b]
−20 to −40	≥2 GΩ	≤200 pA	<10	10–20	150–300	slight outward rectification

Values taken from 18 cells from two independent batches of HEK293 cell (source ATCC).
[a] A study by Gentet et al. (2000) shows that the true membrane capacitance of an HEK cell (~1.1 μF/cm^2: as opposed to the approximation given here for a single cell taken from the patch-clamp amplifier), is about 20% greater than that of many classes of cultured neuron (~0.9 μF/cm^2: cortical, spinal, hippocampal) and is on a par with cultured glial cells.
[b] Fig. 3D shows a typical current–voltage plot for a non-transfected, single HEK293 cell.

3. Show characteristic morphology and size—rounded cells are stressed cells and flat cells are more difficult to access with the patch electrode.
4. Should 'snap' cleanly onto the end of the recording pipette during the patching procedure (requiring healthy, clean membranes, and also a clean patch pipette). The membranes of unhealthy cells are no longer elastic so do not form good (≥2 GΩ) electrode seals.
5. Will proceed to the whole-cell configuration cleanly upon application of negative pressure and show no more than a 10–15% change in series resistance during the recording window (electrode resistances of 3–5 MΩ seem ideal for this.
6. Possess healthy biophysical characteristics (see Table 3).
7. Generate maximum currents of no more than 2–3 nA for good membrane clamp fidelity.
8. Demonstrate no, or minimal, current 'run-up' or 'run-down' during the recording window (see Section 3.3).
9. Demonstrate the expected current profiles for the expressed receptor (see Section 3.4).
10. Do not have a whole-cell holding current which exceeds 300 pA during the recording period.

3.2. Delivery of intracellular modulators

The HEK cell has similar proportions to a neuronal soma but, not possessing extensive branching structures, its intracellular electrolytes equilibrate with the contents of the patch pipette quite rapidly—for example, chloride loading associated with whole-cell recording from $GABA_{A/C}$ receptors using a KCl-based electrolyte solution in the patch electrode, occurs within the first 2–5 min following membrane breakthrough. We have modelled the diffusional characteristics of various intracellularly acting drugs, such as cAMP which we commonly use to alter the phosphorylation state of the $GABA_A$ receptor. If not membrane permeant, such fast acting modulators of receptor function are included in the recording patch pipette, thus it is imperative to know how much time is required for the modulator to have accessed or equilibrated with the majority of its cellular targets. This is especially important if it is not immediately obvious from raw current traces if there is an effect. Fig. 2A outlines the application of Fick's first law of diffusion for substances present in the patch pipette to achieve equilibrium in the cell by diffusion through the pipette orifice, by considering the parameters involved in defining the diffusion rate for a molecule of known molecular weight. The time constant for diffusion, τ, will be a function of the access resistance, R_A; cell geometry, particularly volume, V; the diffusion coefficient for the molecule being investigated, D; and the resistivity of the patch pipette solution, ρ, according to:

$$\tau = \frac{V \cdot R_A}{\rho \cdot D}$$

Chemical reaction rates for most biological processes are comparatively rapid, and therefore insignificant in the estimation of this diffusion constant. The resitivity, ρ, for a 140 mM chloride patch electrolyte will be approximately 0.6 Ω m, and we can approximately describe the volume of a HEK cell by analogy to two back-to-back cones of 'height' (h) 20 μm and radius (r) 5 μm according to the following equation:

$$V = 2 \cdot \left(\frac{1}{3} \cdot \pi \cdot r^2 \cdot h \right)$$

An approximate diffusion coefficient (D) can be obtained from the standard relationship described in Fig. 2B, knowing the molecular weight of the test compound. This plot is derived from published data for diffusion coefficients covering a range of molecules of defined molecular weight. Here D is related to the inverse of the square root of the molecular weight. As an example of a calculation of τ, we will use cycloheximide (the eukaryotic protein synthesis inhibitor), molecular weight 281 ($D \approx 5.2 \times 10^{-10}$ m^2/s), diffusing through an electrode of access resistance 5×10^6 Ω and solution resistivity of 0.6 Ωm, into a cell of volume 1000×10^{-18} m^3, the τ would be calculated as follows:

$$\tau = \frac{1000 \times 10^{-18} \cdot 5 \times 10^6}{0.6 \cdot 5.2 \times 10^{-10}}$$

$$\tau \approx 16.0\,\text{s}$$

This value is the time taken to attain 63% of the maximum concentration inside the cell, thus waiting 2–3 times the value of τ should ensure that the molecule has virtually attained equilibrium. The rate constant can be approximately scaled depending on the size of molecule introduced into the HEK cell, thus effectors such as a large inhibitor peptide (i.e. kDa molecular weight) will take significantly longer to access its

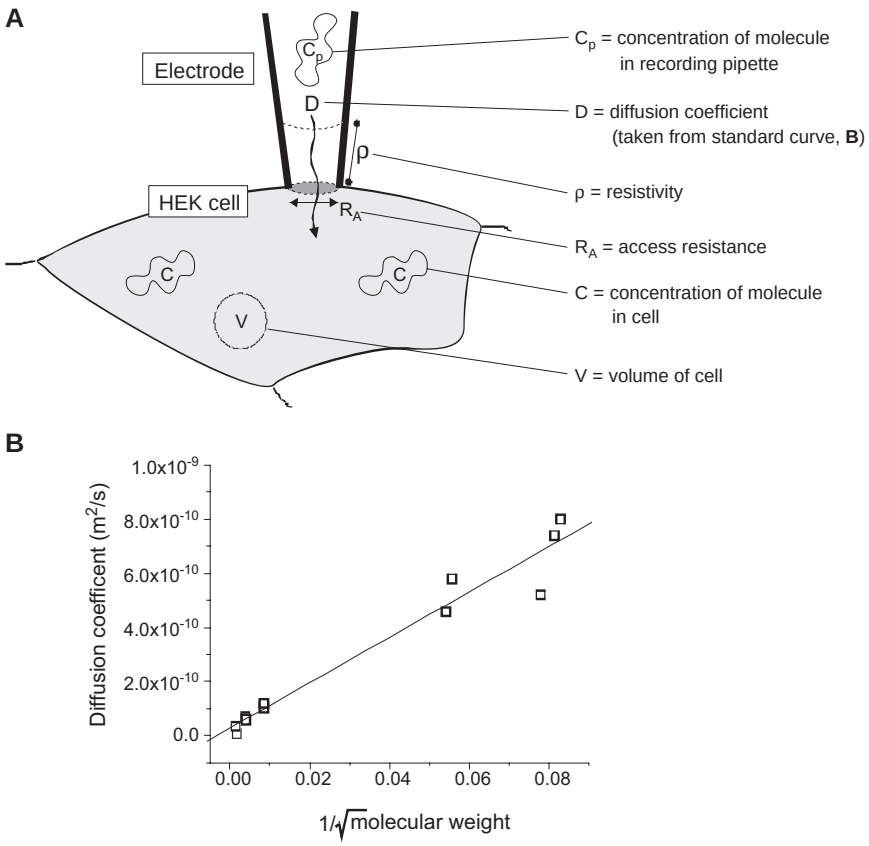

Fig. 2. Parameters used in the calculation of diffusion rates for intracellular effectors of LGIC receptor function. A, Principal parameters used to describe the passage of an effector molecule into the interior of a recorded HEK cell. Explanations of how such parameters are generated can be found in the text. The volume of the HEK cell is modeled on the scenario of two back-to-back cones which approximate to the morphology of a healthy cell. The diffusion coefficient, *D*, can be estimated from its molecular weight, using the approximate linear relationship represented in B. This standard relationship has been derived from a disparate series of known molecular weight molecules and their experimentally determined diffusion coefficients. This figure has been modified from the original (Thomas & Smart, 2002).

target. This estimation ignores the influence of any cellular obstacles to free diffusion (e.g. charge–charge interactions, or intracellular buffering) which will influence the transition of an effector to its target.

3.3. Whole-cell and single channel currents

Once the 'compartments' represented by the pipette and cell are equilibrated drugs can be applied effectively via a modified U-tube perfusion system (see Section 3.4). Some expressing HEK cells are prone to current instability especially in the initial stages of whole-cell break-through. Though the reason for this is unknown (it may be agonist-induced and related to receptor internalization, or mobilization into the membrane of receptor protein, or due to re-equilibration of diffusible factors which alter phosphorylation states of proteins), it manifests as either a run-up or run-down in current amplitude (for the same concentration of drug). The change in current amplitude can be as high as 50% with little or no change in R_s. Though variable in its severity, we find this to be a characteristic of perhaps 10% of cells, but the problem can be addressed to increase the viability of what otherwise might be regarded as unsuitable cells. The strategy to overcome this is to initially monitor a series (3 to 5) of control drug doses in quick succession to establish a control current level, then apply up to two or three different test treatments of the same duration, before returning to monitor the control dose current. This series is repeated for the whole experiment. If run-up or run-down is consistent, then it is possible to extrapolate between control currents to calculate a 'local' mean control current to which the test currents are compared. If whole-cell currents are stable the period between control drug applications can be extended. If the cell does not exhibit this behaviour in the first 10 min after perforation then it is considered stable and would therefore be appropriate for experiments involving phenomena such as receptor recycling/stability or factors which affect these.

It may be that dialysis of the cell contents (e.g. signaling factors) into the patch pipette may contribute to the instability of the whole cell current. Use of the perforated patch technique (i.e. nystatin or amphotericin included in the recording electrode) reduces the likelihood of this, allowing free diffusion of only the smallest electrolytes. However, the perforated patch will tend to increase the series resistance between the electrode and ground, and consequently increase the possibility of voltage clamp errors

if the expression levels of membrane receptor are high in the HEK cell. In such circumstances it is prudent to reduce the size of the current driving force for the principal permeant ion by substitution, in the physiological saline solution, with an impermeant molecule such as sucrose to maintain osmolarity. Alternatively, the cell's holding potential could be adjusted nearer to the current response reversal potential.

The HEK cell is also amenable to low noise single channel current recording, despite the invaginated nature of its cell surface (see Fig. 1, A3). As with all single channel recording, success depends on the use of higher resistance (>10 mΩ), thick-walled glass electrodes, insulated by Sygard coating, that can be used in all cell-attached or excised patch configurations. Contrary to the whole-cell lifting situation (see Section 3.4), the HEK cell ideally requires to be stuck firmly to the coverslip which may require the use of a collagen–poly-lysine mixed substrata. Patch excision is thought to flatten out the fine filopodia of the HEK cell membrane (Gentet, Stuart, & Clemens, 2000) though this probably does not benefit drug access to an already small surface area. The expressed αβγ $GABA_A$ receptor demonstrates GABA-evoked activity producing channel currents of 1–2 pA of approximately 26 pS conductance (Fig. 3A). Bursting activity is typical, the duration and frequency increasing with agonist concentration. Sub-conductance states are also evident. Generally, excised patches can be stable for between 15 and 30 min after which time seal breakdown becomes problematical.

However, for this type of recording, the efficient expression of receptors often means that patches will contain more than one active channel. In this multi-channel patch situation, application of agonist induces the simultaneous opening of multiple channels causing the summation of their currents. These multiple channels then flicker between open and shut states as they desensitize producing prolonged desensitizing current profiles (Fig. 3B). Multi-channel patches can be avoided at the transfection stage by diluting the amount of 'active' vector (i.e. containing the

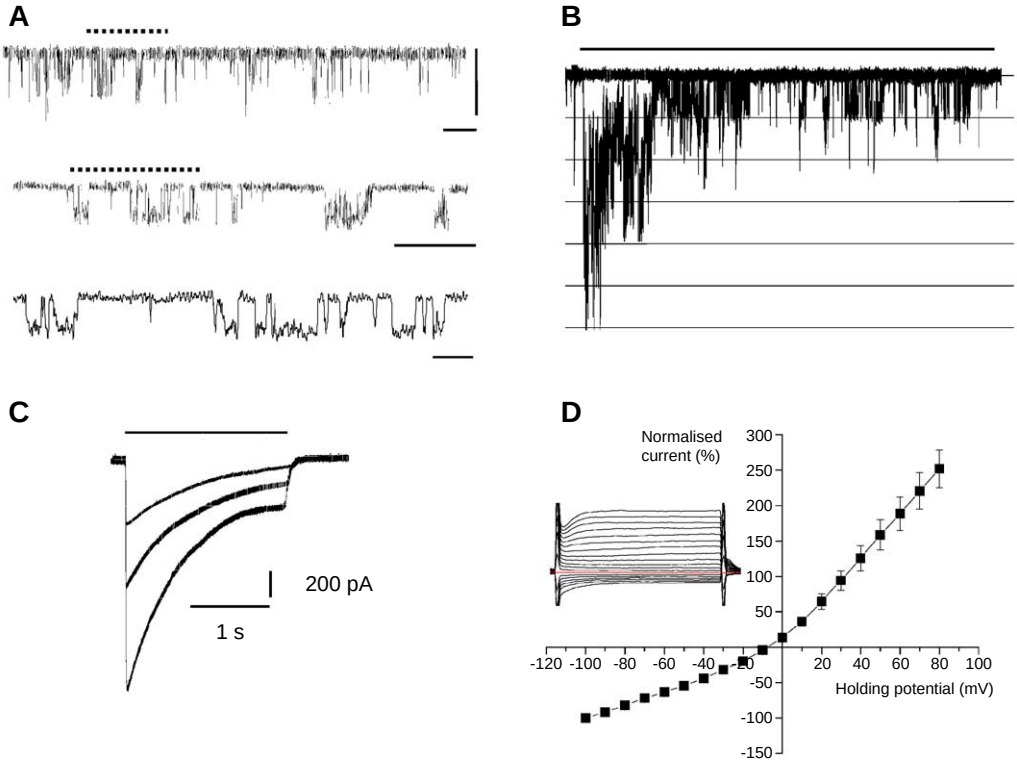

Fig. 3. Current records from HEK 293 cells. A, Excised membrane patches pulled from an HEK cell transfected with α1β2γ2 $GABA_A$ receptor subunits exhibit characteristic single channel currents in the presence of 1 μM GABA. Channels typically have a 1–2 pA current (vertical scale bar=2 pA) and exhibit bursts of openings (downward deflections) which can be sustained for tens of milliseconds (horizontal scale bar=500, 100 and 10 ms for the top, middle and bottom traces, respectively). The dotted lines represent those regions of the current record expanded to form the trace immediately below it. B, Expression (of the same receptor construct as in A) is often so high that attempts to pull a single channel patch can result in a multi-channel as shown here, in which many channels are present in the excised patch and are activated simultaneously upon application of the agonist (black bar). These multiple events (represented by the horizontal lines indicating the unitary conductance levels) eventually desensitise in the continued presence of the agonist. C, Whole-cell current profiles of 1, 10 and 30 μM GABA-activated events from a HEK cell expressing the same construct as in A. Receptor activation times are in the millisecond timescale, though the current rapidly desensitizes in the continued presence of agonist, reaching a steady-state current level before rapid deactivation following wash-out of the drug. D, Current–voltage relationship (n=6) for non-transfected, single HEK cells clamped at the range of voltages indicated by the normalized points. The relationship shows clear outward rectification and the current reverses at roughly −10 mV for the mixed population of voltage sensitive channels endogenous to the HEK cell using a KCl (120 mM) based patch pipette solution. Inset shows representative current profiles for the same holding potentials (negative at the bottom, positive at the top) and associated capacitance transients.

gene for expression) by including a competing 'empty' vector in the transfection mix until adequate receptor expression is achieved (i.e. not too high, but also not too low such that multiple patching attempts have to be made to find a single receptor). It is not sufficient to simply reduce the overall amount of vector/DNA in the transfection as there seems to be a finite amount required for successful expression. An interesting study by Gentet et al. (2000), in which HEK cells were transfected with human Glycine receptor (GlyR) α1 subunit, estimates that transfection by electroporation introduces an average of 20,000 GlyR channels into the whole cell surface. Given the physical dimensions of the LGIC family (typically 8 nm diameter for the muscle nAChR; Unwin, 1993), this number of receptors would theoretically occupy only 0.3% of the surface area of either a 10 μm-diameter HEK cell, or two back-to-back 20 μm cones of base diameter 10 μm, as used in the calculation in Section 3.2. Consequently, the typical LGIC densities in a HEK cell may be of the order of 60 receptors per μm^2, assuming uniform unclustered distribution—a figure which accounts for all the additional area associated with filopodia.

3.4. Drug delivery

In order to achieve near concurrent activation and opening of as many receptors in the HEK cell as possible, the perfusion system must be capable of delivering drug to all targets in the low millisecond timescale. Inevitably, simultaneous activation of a surface receptor population will not be feasible even with the fastest of solution switching devices, but this drug effect latency can be reduced by employing a number of techniques. If whole-cell currents are being recorded then the latency will be at its most extreme but can be minimized by initially choosing smaller cells (this should be verified with capacitance readings from the amplifier) and, if possible, lifting these cells from the dish thus causing the cell to round-up, effectively making the whole of the surface area more accessible to drug. Cell lifting requires that the HEK cell attachment to the coverslip substrata is tenuous, therefore titration of the effective concentration of poly-l-lysine substrata (or equivalent, i.e. poly-D-lysine or poly-ornithine) is necessary. This can be quite arbitrary but start with 50 μg/ml poly-l-lysine and dilute as necessary, though the seeding density may have to be increased to account for the loss of mechanically dislodged cells due to poor attachment. It is also imperative that high resistance seals with the patch electrode are routinely obtained which means cleaning the glass in methanol and storing in a dust-free vessel, but certainly ensuring the cell has a healthy, relatively smooth membrane and minimizing the amount of time the patch electrode is in the bath solution before contacting the cell. The cell lifting technique should not be used for prolonged periods of recording due to the initial and subsequent stress placed on the cell membrane during the transient lifting procedure and mechanical disruption during drug application. Indeed, these procedures may increase cell capacitance by altering the number or distribution of fine filopodia over the HEK cell surface (Gentet et al., 2000).

To apply drugs uniformly to the cell we employ a U-tube system constructed from borosilicate electrode glass which works by forcing drug out through a 30–50 μm hole under gravity via a system of controllable solenoids. Drug onset times are generally of the order of 50–100 ms. Fig. 3C demonstrates the typical response profile of a whole-cell current recorded from a HEK cell expressing α1β2γ2 $GABA_A$ receptors and clearly shows the activation, desensitization, steady-state equilibrium, and deactivation stages. The perfusion system relies on an auxiliary tube placed near to the U-tube which courses physiological saline solution onto the cells in order to rapidly wash away the drug and is a system principally applicable to whole-cell current recording (Thomas & Smart, 2002).

4. Pharmacology

4.1. Advantages

The fidelity with which HEK cells are able to express exogenous receptor makes these cells amenable to many kinds of transfection procedure, permitting the expression of proteins from many sources and for many purposes. For instance, HEK cells have been used to express a wide variety of proteins important to the neuropharmacologist: voltage-sensitive ion channels (sodium, potassium, calcium), G-protein coupled receptors, ligand-gated ion channels, post-synaptic density proteins (PSD95, gephyrin, collibystin) besides many others. At roughly 20–30 μm in length, the cell is a suitable size for most single electrode electrophysiological manipulations from whole-cell recordings to cell-attached or excised patch-clamping, though remains small enough to maintain voltage or current clamp fidelity, unlike the much larger *Xenopus* oocyte. Drug perfusion treatments are accessible to all parts of the cell in the sub-to-low milli-second time scale (depending on method), and cell currents are sustained and will generally be stable for up to 3 h offering a large recording window and the opportunity for many types of manipulation (i.e. rapid and complete analysis of structure–function studies). Peak currents can be obtained with application times of less than a second, thus multiple concentrations of drug can be applied sequentially at roughly 30 s intervals (allowing time for the receptor to recover to its basal state), making it possible to perform 8–10 point concentration–response curves in 10–15 min. Complimentary biochemical analyses are also possible as the HEK cell will express sufficient protein for good signal to noise detection levels in radioligand binding assays, western blotting and immunocytochemical staining. The large amounts of material required for such biochemical manipulations are possible because of low maintenance costs and the rapidity of cell division (24–36 h) for this cell type.

4.2. Disadvantages

There are two principal problems with using this cell type for ion channel electrophysiology studies, though they are far from insurmountable. The first has already been alluded to and concerns the presence of endogenous receptors and channels constitutively expressed by this cell. This may influence the use of this cell type in expressing certain types of exogenous protein, though often expression levels of introduced protein are so high that endogenous proteins are overwhelmed at the membrane surface. The converse is also true for those studying, say, neuronal receptor channels in this epithelial cell derivative, in that many of the neuronal proteins normally involved in receptor trafficking, anchoring and endocytosis will be absent (though this could, on occasions, be an advantage), as will the absence of regenerative currents which characterise neuronal excitability. In a recent study by Mercik et al. (2003) a comparison between $\alpha1\beta2\gamma2$ $GABA_A$ receptors (derived from the same clones), expressed in HEK293 and QT6 cells derived from quail fibroblasts, reported quite different desensitisation kinetics between the cell types following ultra fast GABA application. This difference was in part attributed to the lack of any endogenous receptor anchoring protein, GABARAP, in QT6 cells, as well as differences between the endogenous modulatory systems in these two cell types.

The other significant, but again surmountable, problem associated with HEK cell use concerns the rate of cell division. Because the cells are able to divide in about a day, clusters form quickly. Electrically coupled cells increase the volume (space) which has to be clamped by the recording amplifier, introducing errors which tend to underestimate current amplitudes and are exacerbated as currents increase. This problem is overcome by appropriate single cell (or cell couplets if necessary) choices for recording (see Fig. 1B), or by having rolling transfections every other day.

The potential problem of cell-to-cell variability in data obtained from stable HEK cell clones, as highlighted in Section 1.1 using the WSS-1 cell line as an example, has been thoroughly investigated in a study by Babnigg et al. (2000) targeting a calcium signalling pathway. These authors, besides others in different cell systems, had previously observed large cell-to-cell variations in Ca^{2+} signalling in human fibroblasts, a variation not attributable to the different stages of the cell cycle. Having identified four trp (transient receptor potential) homologues endogenous to the HEK cell, two of which (trp1 and 3) are found to be important mediators of Store Operated Calcium Entry (SOCE), these authors used statistical repetitive sampling methods to establish the degree of difference in the levels of SOCE due to chance variation between single HEK cell clones (via trp knock-out) as compared to the variation seen following transfection with a trp gene. From the analysis of 270 HEK cell clones (each arising from the same cell) a high variation in SOCE values is apparent and is very similar to that seen for a population of 2700 individual cells from the parent population. The outcome of the study, in terms of its impact on electrophysiologists who generally obtain data from a relatively small number of transfected cells, is that variability is inevitable and very high on any given coverslip (at least for this example of a signalling pathway). However, given the impracticality of measuring from hundreds of cells to reduce this variability, a practical solution to transfected HEK cell inconsistencies would be to express inducible constructs such that each clonal population of cells could serve as it own internal control.

5. Discussion

Overall, the HEK293 cell provides a robust and reliable platform in which to express receptor proteins and ion channels with high fidelity. These protein production rates are important if all or part of a protein is to be crystallised, hybrid screened for associated molecules, or used in biochemical assays. Though many of these scale-up procedures can be undertaken in yeast cells, because the HEK cell is amenable to rapid amplification of protein product, it too can be used to generate tens of milligrams of protein for harvesting in weeks, a process which is more time consuming in the CHO cell line, for example. Although selected by neurophysiologists for its properties as a non-neuronal cell in order that neuronal proteins can be studied in isolation from their native cellular milieu, it is evident that the adenoviral transformation, which initially generated this cell line, has endowed these cells with facets that reflect a neuronal phenotype. This characteristic has not precluded the HEK293 from being used extensively across many scientific disciplines and being regarded as a first choice vehicle for the expression of many recombinant proteins.

Acknowledgements

We are grateful to Martin Mortensen (UCL) for providing unpublished data for Fig. 3A and B, to Chris Connelly (University of Dundee) for providing scanning electron micrograph images for Fig. 1, A3, and to the MRC and The Wellcome Trust for support.

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