

RESEARCH ARTICLE

[View Article Online](#)
[View Journal](#) | [View Issue](#)

Cite this: *Med. Chem. Commun.*,
2016, 7, 426

Demonstration of the dynamic mass redistribution label-free technology as a useful cell-based pharmacological assay for endogenously expressed GABA_A receptors†

Anders B. Klein, Mia Nittegaard-Nielsen, Julie T. Christensen, Anas Al-Khawaja and Petrine Wellendorph*

Within the continuous quest for the discovery of pharmacologically interesting compounds, the development of new and superior drug screening assays is desired. In recent years, the use of label-free techniques has paved the way for an alternative high-throughput screening method. An example is the Epic® optical-based biosensor that relies on dynamic mass redistribution (DMR) for detection. So far, DMR assays have been mostly used to study G protein-coupled receptor (GPCR) pharmacology. Here, we demonstrate the utility of this assay for investigating ligand-gated ion channel receptors. Using the immortalized IMR-32 neuroblastoma cell line, which expresses relatively high levels of several endogenous GABA_A receptor subunits, we show that GABA produces concentration-dependent cellular responses that can be measured and quantified in real-time. With the aid of the GABA_A receptor-specific agonist muscimol and the selective antagonists gabazine and bicuculline, we confirm that the data corresponds to that of a GABA_A receptor. Based on quantitative real-time PCR measurements, the subunits α_3 , α_5 , β_3 and θ are the most likely candidates for integration into functional receptors. Our demonstration that label-free methods such as the Epic technology can be used to characterize endogenous GABA_A receptors in the IMR-32 cell line is exemplary for the superfamily of ligand-gated ion channel receptors, and holds interesting perspectives in relation to identifying novel mechanisms of action.

Received 1st October 2015,
Accepted 17th November 2015

DOI: 10.1039/c5md00442j

www.rsc.org/medchemcomm

1. Introduction

γ -Aminobutyric acid type A (GABA_A) receptors in the mammalian brain are pentameric ligand-gated ion channels with an impressive heterogeneity. Due to the existence of 19 different subunits, a variety of receptors with distinct pharmacology and anatomical localization exists.¹ The receptors are important drug targets in the CNS. They are involved in anxiety, anaesthesia and sleep, and mediate fast synaptic transmission that is mainly inhibitory by nature.^{2,3} Traditionally, GABA_A receptor pharmacology research has been performed using electrophysiology and often in *Xenopus laevis* oocytes or mammalian cells overexpressing recombinant GABA_A receptor subtypes.⁴ Whereas such measurements provide detailed knowledge about kinetics and cooperativity, it is a labour-

intensive and low-throughput technique when it comes to compound screening. Furthermore, it fails to give information about the downstream cellular pathways linked to ion channel activation. This has warranted a need for new pharmacological assays to perform reliable and high-throughput drug discovery for GABA_A receptor ligands. The introduction of label-free cell-based assays provides an unresolved potential for finding new GABA_A receptor ligands in a fast and reliable manner and using a holistic readout of cell function. In the current study, we specifically set out to investigate whether label-free cell-based assay using the Epic instrument measuring DMR can be reliably used to study endogenous GABA_A receptor pharmacology in the immortalized human neuroblastoma cell line IMR-32.

As the name infers, label-free assays provide a highly sensitive means to evaluate live cellular responses to ligands in intact cells without addition of an exogenous label. One of the current-generation label-free platforms is the Epic® system developed by Corning Inc., which is based on optical biosensors, but other instruments or biosensors, such as electrical impedance, can also be used for label-free assays.⁵ In this

Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Fruebjergvej 3, DK-2100 Copenhagen, Denmark. E-mail: pw@sund.ku.dk

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5md00442j



assay, multiwell microplates (384 or 96-well), with optical sensors integrated into the bottom, are used to monitor the translocation of cellular mass in response to ligand application. This cellular response is referred to as dynamic mass redistribution (DMR).^{6,7} Most studies on DMR measurements have been performed with G protein-coupled receptors (GPCRs) using both recombinant systems and endogenous models, typically with the aim of framing the signaling pathways of these,^{7–11} but have also been applied for studying protein–protein interactions with GPCRs, *e.g.* potassium channels.¹²

Here, we demonstrate the utility of the DMR technology using the Epic instrument to successfully measure pharmacological responses from GABA_A receptors endogenously expressed in the IMR-32 neuroblastoma cell line.

2. Experimental procedures

2.1. Chemical compounds

γ -Aminobutyric acid (GABA), gabazine (SR 95531) hydrobromide, bicuculline methbromide and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich (St. Louis, MO, USA), (*R,S*)-baclofen was from Abcam Biochemicals (Cambridge, UK), 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP) was a gift from H. Lundbeck A/S, and CGP36742 was a gift from Dr. Wolfgang Froestl, AC Immune. Muscimol was synthesized in-house.¹³ Structures are given in Fig. 1.

2.2. Cell culturing

IMR-32 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, Paisley, UK). The growth medium contained 10% fetal bovine serum (FBS), penicillin (100 units per mL) and streptomycin (100 μ g mL⁻¹), all from Invitrogen. Cells were kept at 37 °C in a humidified atmosphere with 5% CO₂.

2.3. Epic DMR assay

The day before the assay, IMR-32 cells were seeded into an Epic 384-well fibronectin-coated glass microplate (Corning, New York, NY, USA) at a density of 15 000 cells per well. The plate was left for 30 minutes at room temperature and then incubated at 37 °C in a humidified atmosphere with 5% CO₂ overnight (approximately 18 hours). On the day of assay, growth media was removed, and cells were washed three times with assay buffer (Hank's Balanced Salt Solution (HBSS) supplemented with 20 mM 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES), 1 mM CaCl₂ and 1 mM MgCl₂, pH 7.4) using a 24-channel wand suction device (for 384 well format) (V&P Scientific, Inc., San Diego, CA, USA). Cells were then allowed to equilibrate in assay buffer for 1–2 hours at room temperature in the Epic Benchtop System (Corning) until the change during the last 2.5 minutes was less than 5 pm. Up to 0.5% dimethylsulfoxide (DMSO) was added to the buffer if the compound was dissolved in this solvent to avoid variation in buffer composition upon addition of compound. The assay was initiated with 5 minutes of baseline recording after which compounds were added. In experiments with antagonists, GABA was added 10 minutes after application of the antagonist. DMR signals were recorded for 2–3 hours, and data analyzed and exported with the Epic Analyzer Software (Corning). All DMR signals were background corrected.

2.4. RNA extraction, reverse transcription and quantitative PCR (qPCR)

IMR-32 cells were grown to approximately 90% confluency in 10 cm dishes. After discarding the cell media, cells were washed once in phosphate-buffered saline (PBS) (Invitrogen) and total RNA extracted using PureLink® RNA mini kit from Ambion (Thermo Fisher Scientific, Waltham, MA, USA) and following treated with DNase using Turbo DNA-free kit (Ambion), all according to the manufacturer's protocol. The

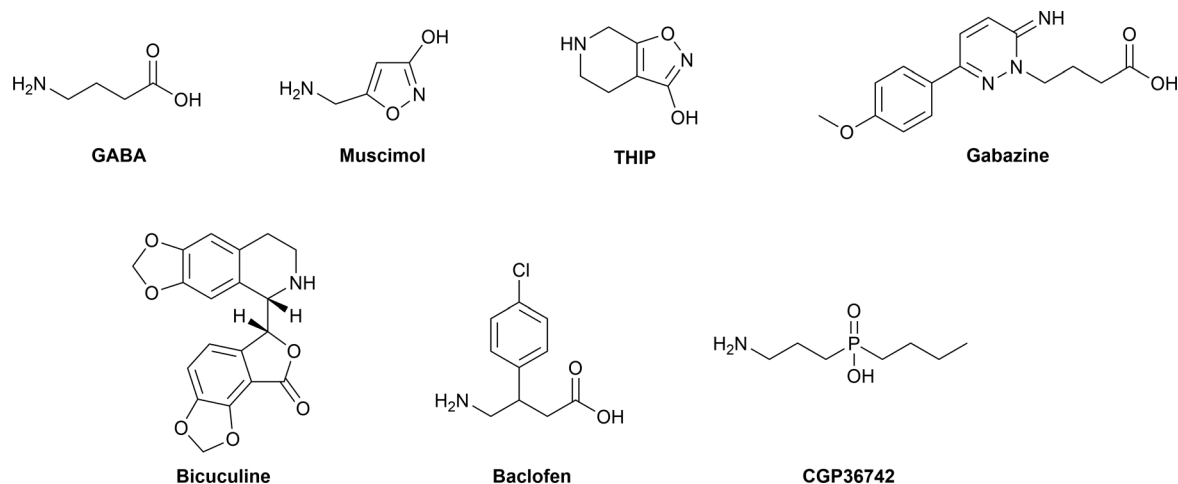


Fig. 1 Structures of compounds used to study GABA_A receptor pharmacology in IMR-32 neuroblastoma cells.



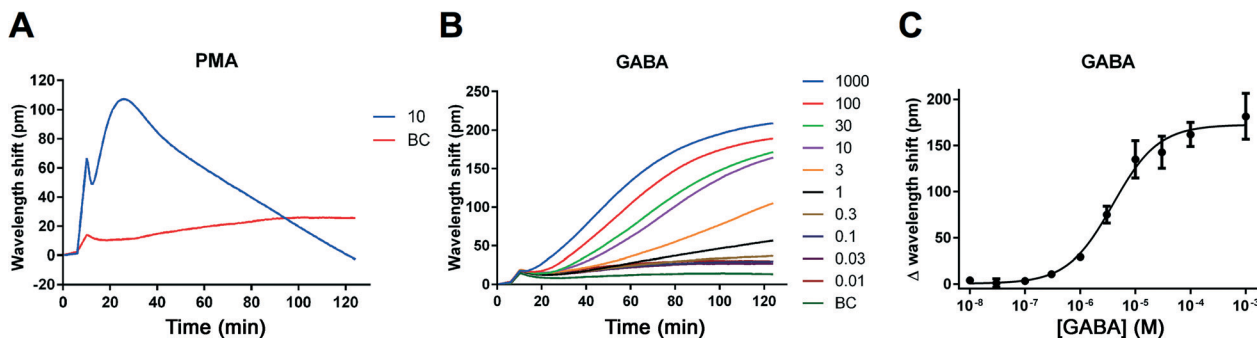


Fig. 2 Ligand-induced DMR signals measured in IMR-32 cells. Kinetic traces after application of the PKC activator, PMA (10 μ M) (A) and increasing concentrations of GABA as a function of time (concentrations given in μ M) (B). Results are shown as means of quadruplicate (A) or triplicate (B) measurements from a single representative experiment. At least four additional experiments gave similar results. GABA responses from (B) at 120 minutes plotted against increasing concentrations of GABA (C). Results are shown as means \pm standard deviations of triplicate measurements. The derived EC_{50} value for GABA is stated in Table 1. BC, buffer control.

reverse transcription was performed using qScriptTM cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA) on a standard PCR machine (25 $^{\circ}$ C for 5 min, 42 $^{\circ}$ C for 30 min, 85 $^{\circ}$ C for 5 min) and cDNA stored at -20° C until further processing.

qPCR was performed in 96-well plates (Agilent Technologies, Santa Clara, CA, USA) mixing PerfeCTa SYBR Green FastMix (Quanta Biosciences), nuclease free water (Qiagen, West Sussex, UK), and primers (TAG Copenhagen A/S (Copenhagen, Denmark)). The PCR was performed with an initial denaturation step of 95 $^{\circ}$ C for 30 s, followed by 40 cycles of 5 s at 95 $^{\circ}$ C, 60 $^{\circ}$ C for 15 seconds and 72 $^{\circ}$ C for 10 s. To assure single-product amplification, a dissociation curve analysis was performed consisting of 60 s at 95 $^{\circ}$ C, 30 s at 55 $^{\circ}$ C and 30 s at 95 $^{\circ}$ C. The qPCR was performed using the Agilent Mx3005P qPCR system (Agilent Technologies), and the corresponding MxPro software was used to determine the Ct values. The Δ Ct values were calculated using $2^{(\text{reference Ct} - \text{target Ct})}$ according to Schmittgen & Livak.¹⁴

2.5. Data analysis

Data and statistical analysis were performed in GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA), and agonist curves were fitted by nonlinear regression using the equation for sigmoidal concentration-response with variable slope:

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(\log EC_{50} - X) \times \text{HillSlope}}}$$

Here, Y is the response, X is the logarithm of the concentration, Top and Bottom are the plateaus in the same unit as Y , EC_{50} is the concentration that gives a response halfway between Bottom and Top, and the HillSlope describes the steepness of the curve.

Similarly, inhibitory curves were fitted by nonlinear regression using the variable slope model:

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(\log IC_{50} - X) \times \text{HillSlope}}}$$

Here, IC_{50} is the concentration that results in a response halfway between Top and Bottom.

3. Results and discussion

3.1. Effects of GABA on IMR-32 cells in the Epic DMR assay

The human neuroblastoma cell line IMR-32 has previously been reported to express functional $GABA_A$ receptors.¹⁵ As our laboratory is generally interested in $GABA_A$ receptors as pharmacological targets and in identifying novel ligands for these, we wished to investigate the possibility of studying endogenous $GABA_A$ receptor signals by means of the label-free DMR technology. The technology has the further advantage that ligands with novel mechanisms of action may be identified. In contrast to traditional electrophysiological methods that focus on the flux of ions across the membrane, the label-free technique measures overall cellular responses and may therefore reveal novel interactions between the ion channel and *e.g.* its effectors.¹⁶

IMR-32 cells were plated in 384-well fibronectin-coated Epic plates approximately 16–20 hours before the assay. To confirm that cells were viable and to confirm assay functionality, we used the compound PMA (a PKC activator) as a positive control.¹⁷ As illustrated in Fig. 2A, a 10 μ M concentration

Table 1 EC_{50} and IC_{50} values for $GABA_A$ receptor agonists and antagonists in IMR-32 cells in the label-free Epic assay. Values are derived from fitted concentration-response curves and are based on at least three independent experiments for each compound (n)

Agonists	EC_{50} (μ M)	$pEC_{50} \pm \text{SEM}$
GABA	2.94	5.58 ± 0.10 ($n = 5$)
Muscimol	2.04	5.77 ± 0.14 ($n = 4$)
Antagonists	IC_{50} (μ M)	$pIC_{50} \pm \text{SEM}$
Bicuculline	16.7	4.80 ± 0.092 ($n = 3$)
Gabazine	7.38	5.34 ± 0.29 ($n = 3$)



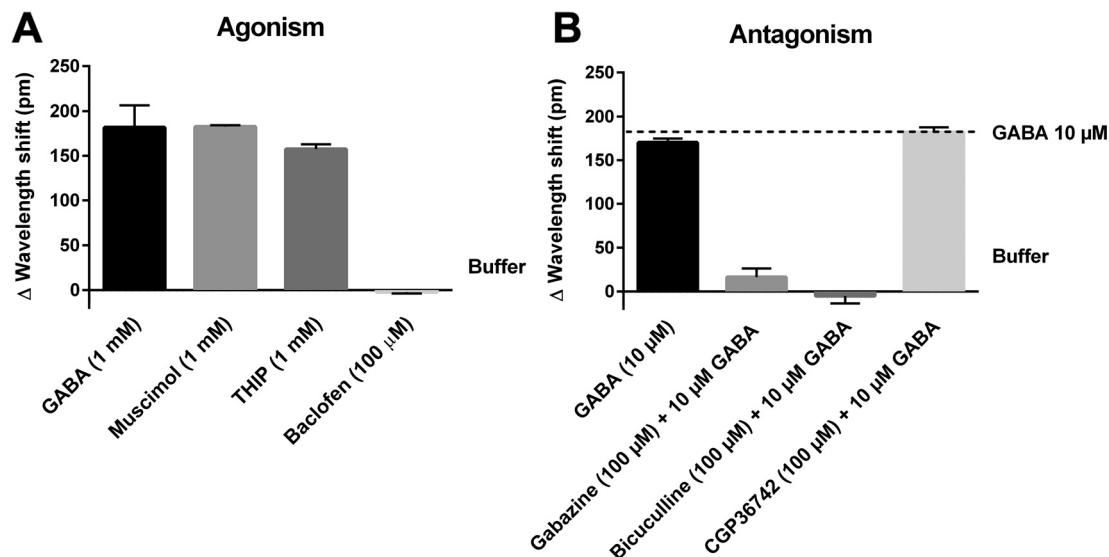


Fig. 3 Validation of GABA_A receptor signals in IMR-32 cells. DMR responses to GABA_A and GABA_B receptor agonists at 1 mM or 100 μM concentrations compared to GABA and buffer levels (A). Effects of GABA_A and GABA_B antagonists at 100 μM concentrations in response to the GABA-induced EC₈₀ concentration (10 μM) (B). Results are shown as means ± standard deviations of triplicate measurements from a single representative experiment (measured at 140 min). Two additional experiments gave similar results.

of PMA induced a robust change in DMR (max. 100 pm signal with a peak after approx. 30 min). We then turned to measuring GABA-induced responses by applying increasing concentrations (0.01–1000 μM) of GABA and recorded for two hours. We observed reproducible and concentration-dependent increases in the DMR kinetic traces that reached plateau after 90–120 min (Fig. 2B). Conversion of the data into sigmoidally shaped concentration–response curves gave a GABA EC₅₀ value of 2.94 μM (Fig. 2C, Table 1).

3.2. Validation of endogenous GABA_A receptor signals in IMR-32 cells using the Epic DMR assay

To confirm that the GABA-induced DMR signals in IMR-32 cells stem from GABA_A receptors and not other GABAergic targets, *e.g.* metabotropic GABA_B receptors, we tested several reference compounds known to be selective for GABA_A and GABA_B receptors. Initially, this was performed using single concentrations (0.1–1 mM) of the GABA_A agonists muscimol and THIP, and the GABA_B agonist baclofen (for structures, see Fig. 1). As would be expected for GABA_A-mediated responses, we observed a large increase in the DMR responses to muscimol and THIP, whereas no effect was seen with baclofen (Fig. 3A). The kinetic profiles of muscimol and THIP had the same shape and time course as GABA (Fig. 2B). Correspondingly, we found that the two GABA_A antagonists, gabazine and bicuculline, but not the GABA_B antagonist, CGP36742,¹⁸ were able to inhibit the GABA EC₈₀ signal (Fig. 3B). In addition, no effect of the antagonists were seen when applied alone (Fig. S1†), demonstrating that no non-specific effects occur at these concentrations. Furthermore, it indicates that the receptors are not constitutively active. The single-concentration determinations verified that GABA_A

receptors are responsible for the DMR signals measured in IMR-32 cells. To further characterize the pharmacology of the employed GABA_A ligands, we made full concentration–response curves. The kinetic traces and corresponding concentration–response curves are shown in Fig. 4, with the obtained potencies collected in Table 1. The DMR signals could be directly transformed into a sigmoidal concentration–response curve for muscimol (Fig. 4A and B), revealing an EC₅₀ value of 2.04 μM (Table 1). Likewise, using 10 μM of GABA, corresponding to EC₈₀, gabazine and bicuculline concentration-dependently inhibited the response (Fig. 4C and E) and yielded IC₅₀ values of 7.38 and 16.7 μM, respectively (Fig. 4D and F; Table 1). For this evaluation we used the late responses (typically 140 min) for quantification. We also noted some smaller peak effects (10–30 pm) after 10–20 min, which were, however very small and not as consistent. In an earlier study, IMR-32 was also shown to be activated by GABA and muscimol in a chloride efflux assay, while baclofen showed no effect,¹⁹ underlining the presence of functional GABA_A receptors in this cell line.

An attribute of DMR label-free assays, which distinguishes them from other cell-based assays, is that they generate a kinetic profile of a given compound. In the current study, we obtained similar-looking kinetic profiles for the tested GABA_A agonists. These developed quite slowly and saturated only after 120–180 min (Fig. 2 and 4). This indicates that, in IMR-32 cells, these ligands all act at the same target, triggering similar intracellular events. The slowly developing DMR kinetic traces obtained for GABA_A receptor agonists in the IMR-32 cells suggest that down-stream cellular effects are taking place after ion channel activation. It highlights the potential of DMR assays to identify signaling pathways induced by ion channel activation that would never be observed in traditional electrophysiological measurements of ion flux.



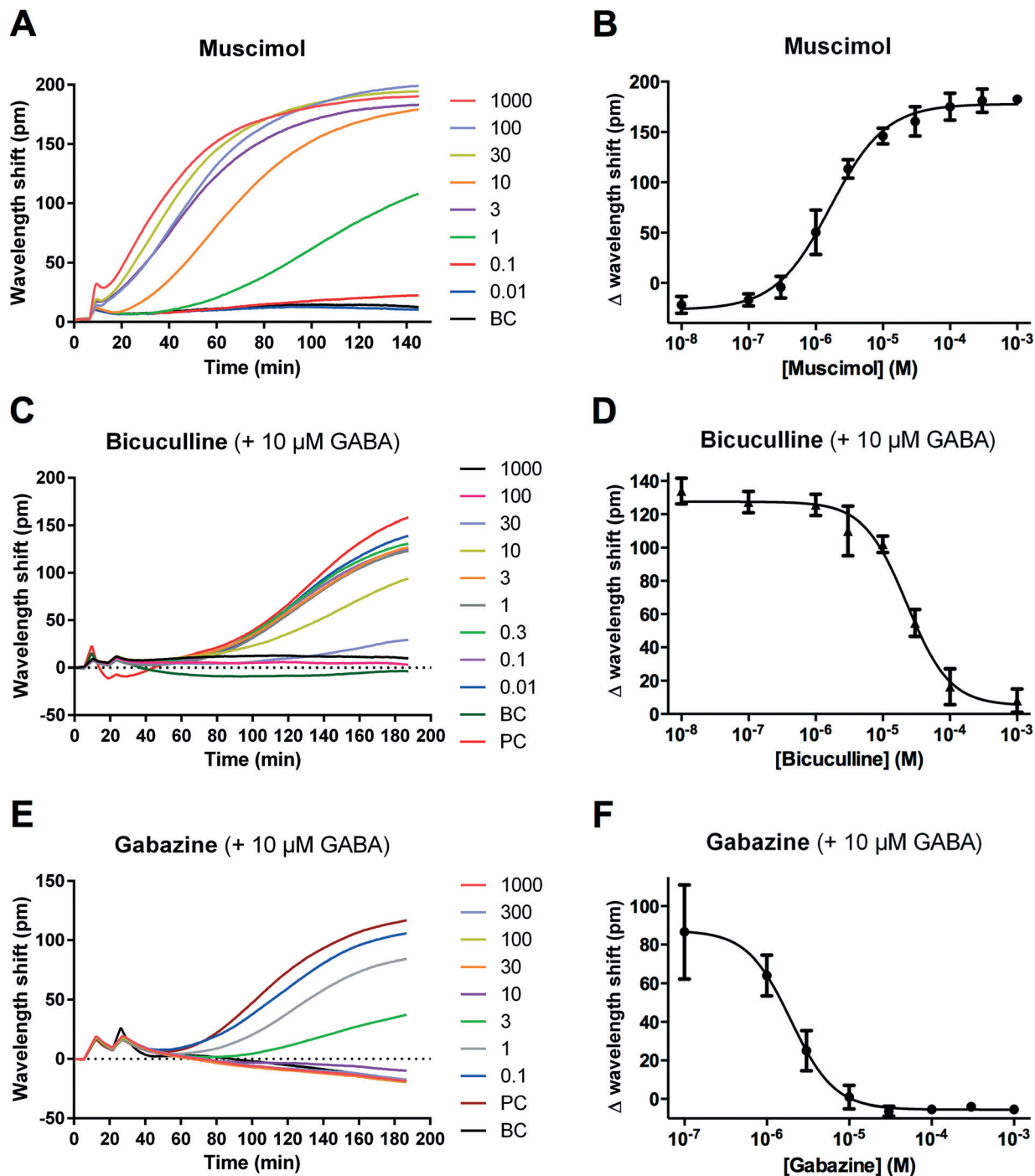


Fig. 4 DMR kinetic traces and concentration-response curves of selected GABA_A receptor ligands in IMR-32 cells. Kinetic traces after application of the agonist muscimol as a function of time (concentrations given as μM) (A). Muscimol responses from (A) at 140 minutes plotted against increasing concentrations of muscimol (B). Kinetic traces after application of increasing concentrations of the antagonists bicuculline (C) and gabazine (E) together with a fixed concentration of 10 μM of GABA ($\sim\text{EC}_{80}$) as a function of time (concentrations given in μM). Antagonist responses from (C) and (E) at 140 minutes plotted against increasing antagonist concentrations (D) and (F), respectively. All results are shown as means of triplicate measurements from a single representative experiment. Derived EC_{50} and IC_{50} values are listed in Table 1. BC, buffer control. PC, positive control (= 10 μM GABA, corresponding to EC_{80}).

Importantly, as reported for several GPCRs, the kinetic profile may be highly dependent on cellular context and should be interpreted with care.⁷ For comparison, a Corning technical report on recombinant $\alpha_1\beta_2\gamma_2$ GABA_A receptors in Wss-1 cells

shows quite a different kinetic profile with a GABA-induced peak after approx. 10–20 min.²⁰ Whether it will be possible to obtain a “profile signature” of GABA_A ligands used to group subtype-specific ligands requires further studies.



Table 2 qPCR primer sequences used to analyze GABA_A receptor subunits in the human neuroblastoma cell line, IMR-32. The reference gene was B2M (beta-2 microglobulin)

Target	Primers	Amplicon size
α_1	F: GGATTGGGAGAGCGTGTAACC R: TGAAACGGGTCCGAACTG	66
α_2	F: GTTCAAGCTGAATGCCCAAT R: ACCTAGAGCCATCAGGAGCA	160
α_3	F: CAACTTGTTCAGTTCATTCCTT R: CTTGTTTGTGTGATTATCATCTTCTTAGG	102
α_4	F: CATGACCACACTAAGCATCAGT R: AAACGCGATAAGGGCCGAAAT	118
α_5	F: CTTCTCGGCGCTGATAGAGT R: CGC TTTTCTTGATCTTGGC	105
α_6	F: ACCCAGAGTGACAATATCAAAAGC R: GGAGTCAGGATGCAAAACAATCT	67
β_1	F: AGGGTAGCTGACCAACTCTGG R: TGTTCCATCAGGATGCCAGTCG	114
β_2	F: GCAGAGTGTCAATGACCATAGT R: TGGCAATGTCAATGTTCTATCCC	137
β_3	F: CCGTTCAAAGAGCGAAAGCAACCG R: TCGCCAATGCCGCTGAGAC	105
γ_1	F: CCTTTTCTTCTGCGGAGTCAA R: CATCTGCCTTATCAACACAGTTTCC	91
γ_2	F: CACAGAAAATGACGGTGTGG R: TCACCCTCAGGAACCTTTTGG	136
γ_3	F: TCCTTTACACTTTGAGGCTCAC R: CCGCCATGATTTCTGGTCAG	176
δ	F: CAATCCTACATGCCCTCCGT R: GAAGTAGACGTCCAGTGCCT	186
ϵ	F: TGGATTCTCACTCTTGCCCTCTA R: GGAGTTCTTCTCATTGATTTCAGCT	107
π	F: CAATTTTGGTGGAGAACCCG R: GCTGTGCGAGGTATATGGTG	110
θ	F: CCAGGGTGACAATTGGCTTAA R: CCCGCAGATGTGAGTCGAT	63
ρ_1	F: TTATTTCCCCGCTACCCGTGAT R: GCACCGTTGTGATACCTAAGG	101
ρ_2	F: TACAGCATGAGGATTACGGT R: CAAAGAACAGGTCTGGGAG	81
ρ_3	F: TGATGCTTTCATGGGTTTCA R: CGCTCACAGCAGTGATGATT	111
B2M	F: CCTGCCGTGTGAACCATGTGACT R: GCGGCATCTTCAAACTCCATGATG	94

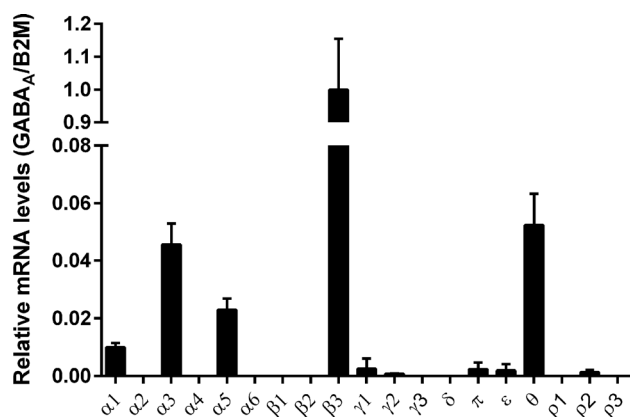


Fig. 5 Relative GABA_A receptor subunit mRNA levels in IMR-32 neuroblastoma cells related to the reference gene, beta-2 microglobulin (B2M). Results are shown as means \pm standard deviations of triplicate measurements from a single representative experiment. Additional experiments gave similar results.

The only other ligand-gated ion channel, reported to date using the label-free technology based on DMR, is the K_{ATP} channel endogenously expressed in C2A cells,²¹ thus in general there is little basis for comparison to our current findings.

3.3. Quantitative PCR analysis of GABA_A receptor subunits in IMR-32 cells

To investigate the relative expression levels of all 19 GABA_A receptor subtypes in IMR-32 cells, qPCR was carried out. To this end, we measured mRNA levels of the GABA_A subunits α_{1-6} , β_{1-3} , γ_{1-3} , δ , π , θ , ϵ and ρ_{1-3} using specific primers (Table 2). The GABA_A subunit displaying the relatively highest expression was the β_3 subunit, while α_3 , α_5 and θ were found to be expressed at 14–33 times lower levels in comparison, but still significantly higher than very low-expressing subunits such as γ_{1-3} and δ (Fig. 5). The relatively high abundance of β_3 , α_3 , α_5 and θ mRNA levels suggests that functional receptors containing these subunits are responsible for the obtained DMR signals in IMR-32 cells. As β_3 is reported to form functional binary and tertiary receptors with either α_3/α_5 , or α_3/γ_5 and θ subunits in *Xenopus* oocytes,^{22,23} it remains unknown which subtype is predominating in IMR-32 cells under the conditions of the assay. The obtained EC₅₀ value for GABA of 2.91 μ M also makes it impossible to discriminate between subtypes, as reported values are quite similar (*i.e.* 4.5 μ M for $\alpha_3\beta_3$ and 3.4 μ M for $\alpha_3\beta_3\theta$, measured in oocytes).²²

Previous reports demonstrate functional GABA_A responses in IMR-32 cells,^{15,24} but the exact subunit combinations are not obvious. Although some studies have presented receptor combinations containing the benzodiazepine site (*e.g.* $\alpha_5\beta_3\gamma_2$),²⁵ others were unable to show any functional benzodiazepine responses.¹⁹ In agreement with these reports is the much higher level of the β_3 subunit in this study. Interestingly, the expression of the θ subunit in IMR-32 cells to significant levels has not been reported before. Future studies using subtype-specific tool compounds and ideally complemented with knock-down studies may be used to reveal the subtype(s) predominantly responsible for the IMR-32 GABA_A-mediated responses and/or the specific pharmacological role of the θ subunit.

Conclusions

As exemplified here, the DMR label-free assay is an attractive assay method for performing quantitative pharmacology of GABA_A receptors. The method may thus prove useful for other ligand-gated ion channels or other non-GPCR targets. Compared to more traditional assays, it gives an information-rich read-out in real-time that correlates to all cellular events. The data from the current study specifically demonstrate the applicability of the label-free assay on endogenously expressed GABA_A receptors in IMR-32 neuroblastoma cells. It provides a potential useful alternative assay with a much higher throughput for pharmacological testing in the search

for novel GABA_A receptor ligands, carried out in a convenient 384-well format. Being label-free, the use of *e.g.* fluorescent labels is avoided, which also extends the application of this technology to fluorescent compound testing.

Acknowledgements

This work was supported by the Lundbeck Foundation (grant R133-A12270), the Danish Research Council, The Aase & Ejnar Danielsen Foundation, The Hørslev Foundation, The Augustinus Foundation and The Carlsberg Foundation. A thank to Prof. Sven Pählman, University of Lund for the IMR-32 cells and to Trine Lind Devantier (Novo Nordisk) for providing the 24-pin suction device.

References

- 1 M. Farrant and Z. Nusser, *Nat. Rev. Neurosci.*, 2005, **6**, 215–229.
- 2 T. I. Saari, M. Uusi-Oukari, J. Ahonen and K. T. Olkkola, *Pharmacol. Rev.*, 2011, **63**, 243–267.
- 3 U. Rudolph and F. Knoflach, *Nat. Rev. Drug Discovery*, 2011, **10**, 685–697.
- 4 E. Sigel and M. E. Steinmann, *J. Biol. Chem.*, 2012, **287**, 40224–40231.
- 5 M. F. Peters, F. Vaillancourt, M. Heroux, M. Valiquette and C. W. Scott, *Assay Drug Dev. Technol.*, 2010, **8**, 219–227.
- 6 Y. Fang, *Expert Opin. Drug Discovery*, 2011, **6**, 1285–1298.
- 7 R. Schröder, J. Schmidt, S. Blättermann, L. Peters, N. Janssen, M. Grundmann, W. Seemann, D. Kaufel, N. Merten, C. Drewke, J. Gomez, G. Milligan, K. Mohr and E. Kostenis, *Nat. Protoc.*, 2011, **6**, 1748–1760.
- 8 M. Grundmann and E. Kostenis, *Methods Mol. Biol.*, 2015, **1272**, 199–213.
- 9 R. Schröder, N. Janssen, J. Schmidt, A. Kebig, N. Merten, S. Hennen, A. Muller, S. Blättermann, M. Mohr-Andra, S. Zahn, J. Wenzel, N. J. Smith, J. Gomez, C. Drewke, G. Milligan, K. Mohr and E. Kostenis, *Nat. Biotechnol.*, 2010, **28**, 943–949.
- 10 S. Pai, F. Verrier, H. Sun, H. Hu, A. M. Ferrie, A. Eshraghi and Y. Fang, *J. Biomol. Screening*, 2012, **17**, 1180–1191.
- 11 S. Hennen, H. Wang, L. Peters, N. Merten, K. Simon, A. Spinrath, S. Blättermann, R. Akkari, R. Schrage, R. Schröder, D. Schulz, C. Vermeiren, K. Zimmermann, S. Kehraus, C. Drewke, A. Pfeifer, G. M. König, K. Mohr, M. Gillard, C. E. Müller, Q. R. Lu, J. Gomez and E. Kostenis, *Sci. Signaling*, 2013, **6**, ra93, DOI: 10.1126/scisignal.2004350.
- 12 M. R. Fleming and L. K. Kaczmarek, *J. Recept. Signal Transduction Res.*, 2009, **29**, 173–181.
- 13 P. Krosgaard-Larsen and S. B. Christensen, *Acta Chem. Scand., Ser. B*, 1976, **30**, 281–282.
- 14 T. D. Schmittgen and K. J. Livak, *Nat. Protoc.*, 2008, **3**, 1101–1108.
- 15 D. W. Sapp and H. H. Yeh, *J. Neurosci. Res.*, 2000, **60**, 504–510.
- 16 J. Comley, *Drug Discov. World*, 2011, 45–62.
- 17 W. S. Liu and C. A. Heckman, *Cell. Signalling*, 1998, **10**, 529–542.
- 18 W. Froestl, S. J. Mickel, R. G. Hall, G. von Sprecher, D. Strub, P. A. Baumann, F. Brugger, C. Gentsch, J. Jaekel and H. R. Olpe, *et al., J. Med. Chem.*, 1995, **38**, 3297–3312.
- 19 S. M. Anderson, R. J. De Souza and A. J. Cross, *Neuropharmacology*, 1993, **32**, 455–460.
- 20 D. H. Randle and A. Gao, *Label-free ion channel assays using the Corning Epic system*, Corning technical report: (http://www.corning.com/media/worldwide/cls/documents/snappshots_156_labelfree_ion_channel_assays_epic_system.pdf).
- 21 H. Sun, Y. Wei, H. Deng, Q. Xiong, M. Li, J. Lahiri and Y. Fang, *Sci. Rep.*, 2014, **4**, 4934.
- 22 M. Mortensen, B. Patel and T. G. Smart, *Front. Cell. Neurosci.*, 2011, **6**, 1.
- 23 N. Karim, P. Wellendorph, N. Absalom, G. A. Johnston, J. R. Hanrahan and M. Chebib, *Amino Acids*, 2013, **44**, 1139–1149.
- 24 X. Zhou and S. S. Smith, *Neuropharmacology*, 2009, **56**, 1041–1053.
- 25 F. Wang, Z. Xu, C. T. Yuen, C. Y. Chow, Y. L. Lui, S. Y. Tsang and H. Xue, *Neuropharmacology*, 2007, **53**, 574–582.

