N-terminal tagging of human P2X7 receptor disturbs calcium influx and dye uptake

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Received: 25 December 2016 / Accepted: 13 December 2017 / Published online: 30 December 2017
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Abstract

The P2X7 receptor is a frequently studied member of the purinergic receptor family signalling via channel opening and membrane pore formation. Fluorescent imaging is an important molecular method for studying cellular receptor expression and localization. Fusion of receptors to fluorescent proteins might cause major functional changes and requires careful functional evaluation such as has been done for the rat P2X7 receptor. This study examines fusion constructs of the human P2X7 receptor. We assessed surface expression, channel opening with calcium influx, and pore formation using YO-PRO-1 dye uptake in response to BzATP stimulation in transfected cells. We found that tagging at the N-terminal of the human P2X7 receptor with the enhanced green fluorescent protein (eGFP) disturbed channel opening and pore formation despite intact surface expression. A triple hemagglutinin (3HA) fused to the N-terminal also disrupted pore formation but not channel opening showing that even a small tag alters the normal function of the receptor. Together, this suggests that in contrast to what has been observed for the rat P2X7 receptor, the human P2X7 receptor contains N-terminal motifs important for signalling that prevent the construction of a functionally active fusion protein.

Keywords Purinergic · ATP · Fluo4 · Flow cytometry

Introduction

The P2X7 receptor belongs to a family of membrane-bound purinergic receptors that sense various extracellular nucleotides. The receptor is expressed in various different cell types primarily in the immune system [1, 2] and less on other cells, e.g. in the nervous system [3], epithelium [4], fibroblasts [5], and bone cells [6] where it is involved in a broad variety of functions.

The P2X7 receptor consists of an intracellular N-terminal, two transmembrane domains linked by an extracellular

domain, and a long intracellular C-terminal. The P2X7 receptor is stimulated by ATP in the micromolar range and the activated P2X7 receptor trimerizes to form a cation channel leading to entry of cations and depolarization of the cell. Upon prolonged activation or at higher concentrations (millimolar range), the receptor forms a non-selective pore allowing entrance of molecules up to 900 Da [7].

The use of fluorescent imaging for monitoring the cellular expression, localization, and trafficking is a useful tool for studying the receptors in response to physiological stimuli. Fluorescent tagging is most often done at the N- or C-terminal part of membrane receptors, although this sometimes leads to malfunctioning receptors. Hence, thorough testing of a receptor fusion construct is necessary. Most studies of the P2X7 receptor involved fusion constructs of rat P2X7 receptor. These investigations apply ion flux measurements, changes in membrane permeability, and surface expression studies to validate the function of chimeric rat P2RX7-eGFP receptor constructs [8–10].

We applied a similar methodical approach and used Fluo4 calcium assay, DNA-binding YO-PRO-1 dye uptake, and flow cytometry to compare human P2X7 receptor tagged at

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the N- or C-terminal to wild-type human P2X7 receptor. This is to our knowledge the first study that examines constructs of human P2X7 receptor N-terminally linked to a fluorescent protein. In contrast to what has been found for the rat P2X7 receptor, N-terminal tagging of the human P2X7 receptor disrupted channel opening and pore formation even though the receptor was normally expressed on the cell surface. Pore formation was also disturbed in human P2X7 tagged with 3HA at the N-terminal showing that the size of the eGFP tag did not alone explain the functional disturbance.

Methods

Cell culturing

A genetically engineered HEK-293 stably expressing human macrophage scavenger receptor for better-adhering properties called GripTite 293 MSR cell line (a kind gift from Søren Gøsgig Faarup Rasmussen, University of Copenhagen) was maintained in DMEM high glucose (#L0102-500, Biowest) supplemented with 10% foetal bovine serum (FBS, #S0115, Biochrom AG), 1% None-Essential Amino Acids (NEAA, #11140-035, Gibco), 0.5–1% Penicillin-Streptomycin (p/s, #DE17-602B, Lonza), and Geneticin® Selective Antibiotic G418 Sulfate (#10131-019, Gibco, Life Technologies) at 37 °C, 5% CO₂, humidified air. Cells were passaged two to three times per week in 1:1 Trypsin (#L0930-100, Biowest)/Versene (#15040-066, Gibco, Life Technologies).

Vector constructs

GripTite cells were transiently transfected with combinations of vectors expressing eGFP, *P2RX7* (NM_002562) in pcDNA3.1 backbone, eGFP-N-*P2RX7* (#EX-Z8319-M29, GeneCopoeia), *P2RX7*-C-eGFP (EX-Z8319-M03, GeneCopoeia), 3HA-N-*P2RX7* (#EX-Z8319-M06, GeneCopoeia), or empty pcDNA3.1 vector. The vector constructs were bought as human wild-type *P2RX7* (NM_002562) in pEZ backbone vector joined at the N- or C-terminal by 17 and 15 nucleotide linker regions, respectively, under the control of CMV promoters. Because all tags were intracellular, they were not expected to interfere with ligand binding. Vectors were transformed into XL1-Blue Competent Cells (#200249, Agilent Technologies) by a short heat pulse and plated onto LB agar (#813550, RegionH Pharmacy) plates with 100 µg/mL ampicillin (#A9518-5G, Sigma-Aldrich). Colonies were amplified in LB medium (#813527, RegionH Pharmacy) containing 100 µg/mL ampicillin before purification with NucleoBond Xtra Midi kit (#740410.50, Macherey-Nagel). The P2X7 receptor coding sequences were sequenced by Eurofins Genomic, Germany. Sequencing of the P2X7 receptor coding nucleotides of eGFP-N-*P2RX7* and

3HA-N-*P2RX7* proved that there were no deleterious errors following sub-cloning (data not shown).

Transient transfection

Transient transfection was carried out either on adherent cells or in loosened cells before seeding. In the first protocol, the cells were seeded as 9.5×10^5 cells/well in 6-well plate and allowed to attach overnight. The following day, mixtures of Lipofectamine® 2000 Transfection Reagent (#11668-019, Invitrogen, Life Technologies) and DNA dissolved in DMEM +2% FBS for a total of 0.13 mg DNA/well and 0.4% Lipofectamine, respectively. Cells were transfected overnight. Transfection medium was removed on the following day and cells were washed in phosphate-buffered saline (PBS) and loosened with Versene and Trypsin before seeding 3×10^5 cells/well in a black clear-bottom 96-well plate and allowed to attach overnight.

For the latter transient transfection protocol, the DNA was mixed in 1:1 ratios in serum-free medium for a total of 20 ng/µL. Lipofectamine was diluted 1:10 in serum-free medium following by mixing with DNA solution to allow complex formation for 5 min at room temperature (RT) before dissolving in serum-free medium to a final concentration of 1 ng/µL total DNA and 0.5% Lipofectamine. Cells were loosened as described above and resuspended in the DNA:Lipofectamine solution at a final concentration of 500,000/mL. Cells in transfection suspension were seeded as 100 µL/well in black optical-bottom 96-well plates or 500 µL/well in 4-well chamber slides (#154526, Nunc, Thermo Scientific). Cells were allowed to attach for 5 h at 37 °C, 5% CO₂, humidified air before changing to medium containing FBS, p/s, and NEAA.

Channel opening with Fluo4 calcium signalling

Cells were washed with washing buffer (Hank's balanced salt solution (HBSS) with Ca²⁺/Mg²⁺ (#14065-049, Gibco), 10 mM HEPES (#15630-080, Gibco), 2 mM probenecid (#P8761, Sigma-Aldrich), pH = 7.4)) and next incubated in loading buffer (2 µM Fluo4 AM (#F14201, Molecular Probes, DMSO), 0.02% pluronic acid F-127 (#P2443, Sigma-Aldrich, DMSO) in washing buffer) for 30 min. Cells were washed and allowed to rest for 10 min in washing buffer before automatic addition of the synthetic ATP analogue, 3'-O-(4-benzoyl)benzoyl-ATP (BzATP, #B6396-25MG, Sigma-Aldrich) and finally, the signal was detected using the NOVOSTar microplate reader system (BMG LABTECH GmbH). Data were plotted as time-response curves and the maximal fluorescence intensity above baseline was calculated relative to baseline ($\Delta F/F_0$). These values were used to calculate the average background from the three lowest tested concentrations showing no response. The normalized calcium

response was calculated by subtracting the average background value from each sample in the dose-response curve.

Pore formation with YO-PRO-1 dye uptake

Twenty-four hours after the medium change of 96-well plate-transfected cells, the cells were washed once in dye buffer prepared as HBSS (#14170112, Gibco, Life Technology) with 0.2% YO-PRO-1® Iodide 491/509, (#Y3603, Life Technologies) and left in the buffer for baseline measurement. Plates were read with the NOVOStar every 2 min at 489/510 nm for 8 min before manually adding a respectable concentration of BzATP along with Triton X-100 as a positive control. F_0 was calculated as the average over cycle 2–5 (2–10 min). Fluorescence intensity at time 0 min was excluded because there was often a slight change in baseline most likely due to temperature adjustment of the plate in the reader. Maximal pore formation was measured 64 min after BzATP addition. Longer stimulation sometimes resulted in cell death leading to cell detachment and loss of signal (data not shown). Pore formation was assessed by calculating the final max fluorescence intensity subtracted the average baseline fluorescence intensity ($F_{\max} - F_0$). Figure 1a shows an example of a large YO-RPO-1 fluorescence intensity response measured in a single well with *P2RX7*-transfected GripTite cells treated with the highest tested dose for pore formation, i.e. 250 μ M BzATP.

Surface expression with flow cytometry

Cells were prepared for flow cytometry by resuspension in PBS + 2 mM EDTA (#840363, RegionH Pharmacy), washed with PBS + 2 mM EDTA, and stained with Fixable Viability Dye e506 (#65-0866-14, eBioscience). Blocking was done in 10% goat serum (#G9023-5ML, Sigma-Aldrich) before staining with 1.5 μ g/mL mouse anti-human P2X7 receptor monoclonal antibody (mAb; [11]) and mouse IgG2b isotype control (#557351, BD Bioscience) followed by 1:250 APC-conjugated goat anti-mouse secondary antibody (#550826, BD Bioscience) diluted in PBS + 2 mM EDTA + 0.5% goat serum. Incubation times were 0.5–1.5 h. The cells were analysed by flow cytometry on a FACSverse (BD Biosciences, USA) and the FACSuite software (BD Biosciences, USA) was used for data analysis. Gating was set at 0.10% for both GFP and P2X7 receptor signals on empty vector control (pcDNA3.1) with isotype staining.

Spectral overlap testing

YO-PRO-1 and Fluo4 have fluorescent spectra that are almost identical to that of eGFP (Semrock SearchLight

Fig. 1 **a** Example of data from pore formation assay showing YO-PRO-1 fluorescence intensity increase over time in a single 96-well with GripTite cells transfected with *P2RX7* + empty vector and stimulated with 250 μ M BzATP. **b** Fluorescence intensity baseline for GripTite cells transfected with *P2RX7* in combination with empty vector (pcDNA3.1) or a vector for eGFP. There was no statistically significant difference between groups when compared in an unpaired two-sided *t* test using GraphPad Prism 6. Data are shown as mean + SEM, $n = 7$ –8 from three independent runs

<http://searchlight.semrock.com/>). Because of the overlapping fluorescence spectra, we worried that the eGFP tag on *P2RX7* would mask the Fluo4 and YO-PRO-1 responses by increasing the baseline and give a false-negative result. To test this, functionally active *P2RX7* was cotransfected with an eGFP vector to see if the fluorescence intensity measurement would be saturated by the eGFP signal. In the YO-PRO-1 assay, there was no significant difference in the mean baseline level between cells expressing eGFP along with *P2RX7* and cells only expressing *P2RX7* (Fig. 1b) suggesting that the signal from eGFP did not interfere. We, therefore, conclude that the spectral overlap did not interfere and that the assays were sensitive enough to detect BzATP-induced *P2RX7* receptor signalling on top of background fluorescent eGFP cells.

Immunocytochemistry

Twenty-four hours after the medium change of 4-well chamber slides cells were fixed in 4% paraformaldehyde in PBS for 10 min at RT. Cells were washed twice in PBS and permeabilized 30 min at RT in PBS containing 0.1% Triton X-100 (#T8787, Sigma-Aldrich). Blocking was done 1 h in blocking buffer (PBS, 10% donkey serum (#017-000-121 lot: 129348, Jackson ImmunoResearch)). Monoclonal mouse anti-HA (#901513, BioLegend) was dissolved 1:1000 in blocking buffer and left at RT overnight. On the following day, the cells were washed in permeabilization buffer and incubated 1 h at RT in 1:250 donkey anti-mouse Alexa Fluor 488 (#715-545-151, Jackson ImmunoResearch) dissolved in blocking buffer. Wells were washed three times in PBS before mounted with Vectashield containing DAPI (#H-1200, Vector Laboratories). Fluorescence was visualized with a Axiophot microscope (Carl Zeiss Microscopy, Germany) coupled to an AxioCam HR digital camera.

Results

Loss of signalling of N-terminally eGFP-tagged human P2X7 receptor

The calcium signalling properties were compared in N- and C-terminally eGFP-tagged human P2X7 receptor and normal wild-type receptor following stimulation with BzATP. As seen in Fig. 2a, the *P2RX7*-C-eGFP group had a calcium response identical to that of the group expressing the human wild-type receptor. In contrast, the eGFP-N-*P2RX7* group had a similar response to that of cells transfected with an empty vector. The empty vector control showed a small response to 400 μ M BzATP, suggesting that other endogenous purinergic receptors with lower affinity to BzATP are present in GripTite cells (Fig. 2a).

The pore-forming property of eGFP-N-*P2RX7* and *P2RX7*-C-eGFP constructs was compared to human *P2RX7* by measuring YO-PRO-1 dye uptake. There was no increase in YO-PRO-1 fluorescence intensity in eGFP-N-*P2RX7*-transfected cells at any of the tested concentrations (Fig. 2b). At concentrations ranging from 62 μ M BzATP to above the P2X7 receptor pore formation appeared to reach a plateau in wild-type receptor-transfected cells. The dye uptake in the *P2RX7*-C-eGFP receptor group was slightly lower but not significantly different from that of the untagged P2X7 receptor.

P2X7 receptor construct surface expression

We speculated whether the loss of calcium and YO-PRO-1 signalling from the human eGFP-N-*P2RX7* construct was caused by a lack of surface expression of the tagged receptor

Fig. 2 **a** N-terminal tagging of the human P2X7 receptor disturbs calcium signalling and pore formation. Fluo4 calcium signalling in GripTite cells transiently transfected with different vectors expressing *P2RX7* (triangle), C-terminally eGFP-tagged *P2RX7* (*P2RX7*-C-eGFP) (diamond), N-terminally eGFP-tagged *P2RX7* (eGFP-N-*P2RX7*) (circle), and empty vector (cross). Data represents mean $\pm$ SEM, $n = 4-6$ from two independent runs. **b** YO-PRO-1 dye uptake as a measure of pore formation in GripTite cells transiently transfected with combinations of vector expressing *P2RX7* + empty vector (triangle), *P2RX7* + eGFP (square), *P2RX7*-C-eGFP + empty vector (diamond), and eGFP-N-*P2RX7* + empty vector (circle). *** $p < 0.001$, **** $p < 0.0001$ for *P2RX7* + empty vector vs. eGFP-N-*P2RX7* + empty vector, # $p < 0.05$, ### $p < 0.001$, #### $p < 0.0001$ for *P2RX7* + eGFP vs. eGFP-N-*P2RX7* + empty vector, and \$ $p < 0.05$ for *P2RX7*-C-eGFP + empty vector vs. eGFP-N-*P2RX7* + empty vector analyzed in two-way ANOVA post hoc Tukey's multiple comparisons test at respective concentrations using GraphPad Prism 6. Data represents mean $\pm$ SEM, $n = 3-6$ from six independent runs

due to disruption of the N-terminal signal peptide sequence. To determine the extent of surface expression, flow cytometry using a P2X7 receptor antibody that recognizes the extracellular part of the receptor was carried out. The GFP signal from cells transfected with chimeric protein was used as an indicator of transfection efficiency and it was comparable between

the *P2RX7*-C-eGFP (8.43%; Fig. 3c) and eGFP-N-*P2RX7* (6.73%; Fig. 3e) groups.

When stained with the extracellular P2X7 receptor antibody, 8.10% of *P2RX7*-C-eGFP (Fig. 3d) and 5.32% of eGFP-N-*P2RX7* (Fig. 3f) cells were positive for both eGFP and the APC signal. For *P2RX7*-C-eGFP and eGFP-N-*P2RX7*, 18.6 and 12.9% cells, respectively, were P2X7-positive/eGFP-negative. These cells are falsely eGFP-negative due to technical limitations of the flow cytometer causing only medium sensitivity towards eGFP.

The relative differences in expression efficiencies cannot explain the measured calcium and pore formation responses. If we consider the P2X7 positivity showing approximately 30% for wild-type *P2RX7* (Fig. 3b), 24% for *P2RX7*-C-eGFP (Fig. 3d), and 17% for eGFP-N-*P2RX7* (Fig. 3f), we would still expect a signal (however decreased $F_{\max}$) from the N-terminally tagged receptor because it is expressed on the cell surface. Nevertheless, we observed a complete loss. Hence, both *P2RX7*-C-eGFP and eGFP-N-*P2RX7* were detected of the cell surface, and therefore, the loss of calcium and YO-PRO-1 signals following fluorescent N-terminal tagging was not due to absent surface expression.

Loss of function at the N-terminal also with the small HA tag

To investigate if the size of the fluorescent eGFP molecule was causing the signalling disruption, we tested another vector construct comprising human P2X7 receptor fused at the N-terminal to a smaller triple HA (3HA) tag. Interestingly, this receptor construct also affected dye uptake, whereas calcium influx was still present (Fig. 4a, b). Therefore, the size of the N-terminal tag likely affects the inactivation to some extent. The presence of calcium signalling shows that the P2X7 receptor fused to the 3HA at the N-terminal was present on the surface, and the expression of the 3HA-N-P2X7 protein was also verified by immunocytochemical staining. Cells transfected with the 3HA construct showed clear HA immunoreactivity in contrast to the wild-type P2X7 receptor that showed no staining (Fig. 4c). Hence, even a small tag at the N-terminal of the human P2X7 receptor disrupted normal signalling properties.

Discussion

In this study, we used Fluo4 calcium signalling and DNA-binding YO-PRO-1 dye uptake measurements to study human P2X7 receptor with fluorescent eGFP or a smaller 3HA tag. Both assays showed a disturbed function of human P2X7 when tagged at the N-terminal, whereas the C-terminally eGFP-tagged receptor had signalling properties comparable to those of the wild-type P2X7 receptor. The loss of signal

of the tested eGFP-N-*P2RX7* receptor construct was not due to reduced receptor expression in cells or the absence of surface expression as shown by flow cytometry.

This is in contrast to what has been found for rat P2X7 receptor eGFP constructs. In one study, rat P2X7 receptor with eGFP at the N-terminal transiently expressed in HEK-293 cells was functional in calcium assay induced by BzATP as measured by Fura2 [8]. Cellular localization was examined by confocal spectral laser scanning microscopy and appeared in high-intensity spots around the plasma membrane [8] suggesting that the N-terminal eGFP tag did not affect the cellular localization of the rat P2X7 receptor. In another study, calcium signalling and pore formation were intact for N-terminally eGFP-tagged rat P2X7 receptor in a pIRES2 backbone when tested with Fura2 and YO-PRO-1 in GT1 cells [9]. Furthermore, C- and N-terminal eGFP tagging of rat P2X7 receptor were tested for channel and pore function with a voltage-clamp analysis in injected *Xenopus* oocytes and uptake of ethidium ions in transfected HEK-293 cells, respectively [10]. The N-terminally tagged receptors displayed signalling identical to the wild-type rat P2X7 receptor, whereas the C-terminally tagged receptor showed reduced channel signalling [10]. Both constructs were confirmed to localize in the plasma membrane analogue to the wild-type by confocal microscopy. Hence, the rat P2X7 receptor coupled to eGFP at the N-terminal appears to produce a functionally active protein that displays both channel and pore signalling properties.

Other species differences involving the N-terminal of the P2X7 receptor have been observed for the MAPK inhibitor SB-203580, which was found active only on the wild-type or chimeric P2X7 human-rat/human-mouse receptor constructs expressing the human first 255 amino acids including the N-terminal [13]. It should be mentioned that the eGFP tagging of the human P2X7 receptor could also affect other functional endpoints connected to intracellular motifs such as ERK1/2 or N-linked glycosylation [8, 14] that were not investigated in this paper.

In a very recent study published by Karasawa et al. [12], they examine HEK-293 cells expressing panda P2X7 truncated at the N-terminus (N Δ 1–22). Panda P2X7 receptor is ~85% identical to the human P2X7, whereas rat is only ~80%. They find that the truncated receptor had more than tenfold reduced YO-PRO-1 uptake compared to the full-length receptor. The ion channel signalling property was studied by patch clamp and was diminished by ~50% for N Δ 1–22. Hence, the N-terminus of panda P2X7 contains important residues necessary for normal function and it is possible that an eGFP or 3HA tag fusion to human P2X7 sterically blocks these residues.

We also studied the signalling properties of C-terminally eGFP-tagged human P2X7 receptor and the wild-type receptor and found identical signalling properties. A human *P2RX7*-C-eGFP construct has previously been shown to be

Fig. 3 N- and C-terminally tagged P2X7 receptors are expressed on the cell surface. Flow cytometry dot plots showing transiently transfected GripTite cells with **a** empty vector control (pcDNA3.1), **c** human *P2RX7*-C-eGFP, and **e** human eGFP-N-*P2RX7* stained with isotype control. The right column displays cells transiently transfected with **b** human *P2RX7*, **d** *P2RX7*-C-eGFP, and **f** human eGFP-N-*P2RX7* stained with extracellular P2X7 antibody and detected with an APC-conjugated secondary antibody

fully functional. Milius et al. [15] tested a pEGFP-N1 backbone construct similar to the one used in this paper and found that the tagged receptor elicited a BzATP-evoked signal measured with both patch clamp and PI uptake. Both signals were comparable between the C-terminally tagged human P2X7 and wild-type receptor. This article also investigated the localization of the C-eGFP-coupled P2X7 receptor by flow cytometry and found that most cells positive for GFP were also detected by P2X7 receptor surface antibody. In contrast, another study by Ferrari et al. found that a chimeric receptor of

human P2X7 receptor and GFP at the C-terminus was reported mostly retained intracellularly with little surface expression when expressed in HEK-293 cells [16]. The rise in intracellular calcium following ATP-stimulation has been found decreased for the rat P2X7 receptor when C-terminally linked to GFP [16]. The C-terminal of the mouse P2X7 receptor has been found necessary for proper surface expression by comparing P2X7 receptor chimeric proteins constructed from mouse P2X7 receptor and partial P2X1 and P2X4 receptor sequences [17]. Correct targeting of the rat P2X7 receptor to the basolateral membrane was destroyed after deletion of the C-terminal part of the receptor [18]. Likewise, a truncated mouse P2X7 receptor lacking the C-terminal part was found inefficiently trafficked in the plasma membrane [19]. These data point to the C-terminal part of the murine P2X7 receptor to control correct membrane incorporation. The human P2X7 C-terminal eGFP receptor constructs tested in this study had correct surface expression. Together, this suggests there are

Fig. 4 N-terminal tagging with HA disturbs YO-PRO-1 dye uptake but not calcium signalling. **a** Calcium signalling in GripTite cells transiently transfected with different vectors expressing *P2RX7* (triangle), N-terminally triple HA-tagged *P2RX7* (3HA-N-*P2RX7*) (half-open triangle), and empty vector (cross). Data represent mean $\pm$ SEM, $n = 3$ –4 from two independent runs. **b** YO-PRO-1 dye uptake as a measure of

pore formation in GripTite cells transiently transfected with combinations of a vector expressing *P2RX7* (triangle), 3HA-N-*P2RX7*, or empty vector (cross). Data represent mean $\pm$ SEM, $n = 3$ from three independent runs. **c** HA immunoreactivity (green) in transiently transfected GripTite cells expressing *P2RX7* (left) or 3HA-N-*P2RX7* (right). Nuclei were stained with DAPI (blue). Scale = 20 μm

differences between human and murine P2X7 receptor preventing the developing of a functionally active human P2X7 receptor tagged at the N-terminal position.

Conclusions

This paper examines signalling properties of human P2X7 receptors construct tagged at the N- or C-terminal with eGFP or 3HA. BzATP-evoked calcium influx and YO-RPO-1 dye uptake were tested in cells transfected with the chimeric receptors in comparison with the wild-type P2X7 receptor. The C-terminal eGFP tag did not affect signalling of the receptor. In contrast to what has been observed for the rat P2X7 receptor, the N-terminal tags disturbed the dye uptake properties of human P2X7, whereas ion channel signalling was only affected by the eGFP tag. Hence, inactivation of human P2X7 is assumed to be affected by the size of the N-terminal tag. Incorrect trafficking and membrane localization could not explain the lack of signal for the N-terminal eGFP human P2X7 receptor.

Acknowledgements Great thanks to Helle Kinggaard Lilja-Fischer for many hours spent on calcium signalling optimization and Susanne Syberg for excellent teaching of YO-PRO-1 dye uptake assay. Also, thanks to Martin Fredensborg Rath for the use of his equipment.

Funding information The study was supported financially by the Lundbeck Foundation and FP7-CIG grant 334443.

Compliance with ethical standards

Conflict of interest Karin Dreisig declares that she has no conflict of interest.

Nikolaj Pagh Kristensen declares that he has no conflict of interest.
Maja Wallentin Dommer declares that she has no conflict of interest.
Niklas Rye Jørgensen declares that he has no conflict of interest.
Birgitte Rahbek Kornum declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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