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Epilepsy-associated mutation T226R in Kv1.1 subunit: properties of heterotetrameric Kv1.1(T226R)-Kv1.2 channel

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INTRODUCTION & AIM

Co-expression of the Kv1.1 and Kv1.2 subunits in the cells of the central nervous system results in the predominant formation of heterotetrameric voltage-gated potassium Kv1.1/Kv1.2 channels, which regulate neuronal excitability. An inherited mutation of the Kv1.1 channel, T226R, which is associated with episodic ataxia and epilepsy, disrupts the Kv1.1 channel's activity. The influence of this mutation on the properties of the heterotetrameric Kv1.1/Kv1.2 channel was investigated.

METHODS

Expression plasmids encoding fluorescently labeled mKate2-Kv1.1(T226R) and tandem dimer Kate2-Kv1.1(T226R)-Kv1.2 were constructed and used together with the previously created plasmid for expression of mKate2-Kv1.1-Kv1.2 [Efremenko *et al.*, Cells, 2025] to transfect Neuro-2a cells. Recombinant fluorescent ligand HgTx-GFP was produced [Ignatova *et al.*, Cells, 2024]. Confocal microscopy was used to study the ligand binding and distribution of the channels in the cells. The electrical activity of the heterochannels formed by the tandem dimers was studied using a whole-cell patch-clamp technique.

RESULTS & DISCUSSION

Heterologous expression of mKate2-Kv1.1(T226R) and mKate2-Kv1.1(T226R)-Kv1.2 in the plasma membrane and the ability of the dimers to form heterotetrameric channels with a preserved pore structure were studied by binding the fluorescent ligand HgTx-GFP (Fig.1). mKate2-Kv1.1(T226R) showed a web-like pattern in the cytoplasm of cells and the absence of a detectable localization of channels in the plasma membrane (Fig.1a). mKate2-Kv1.1(T226R)-1.2 concatemers demonstrated both cytoplasmic and considerable membrane presentation (Fig. 1c), very similar to that of the mKate2-Kv1.1 (Fig. 1e) and mKate2-Kv1.1-1.2 (Fig. 1g) channels.
The addition to cells of HgTx-GFP, a fluorescent high-affinity ligand of Kv1.1 and Kv1.2 channels [Ignatova *et al.*, Cells, 2024], did not lead to staining of the plasma membrane in the cells expressing mKate2-Kv1.1(T226R) channel (Fig.1b), confirming the absence of its membrane expression. For mKate2-Kv1.1(T226R)-1.2 heterochannels, membrane expression was confirmed by HgTx-GFP binding.

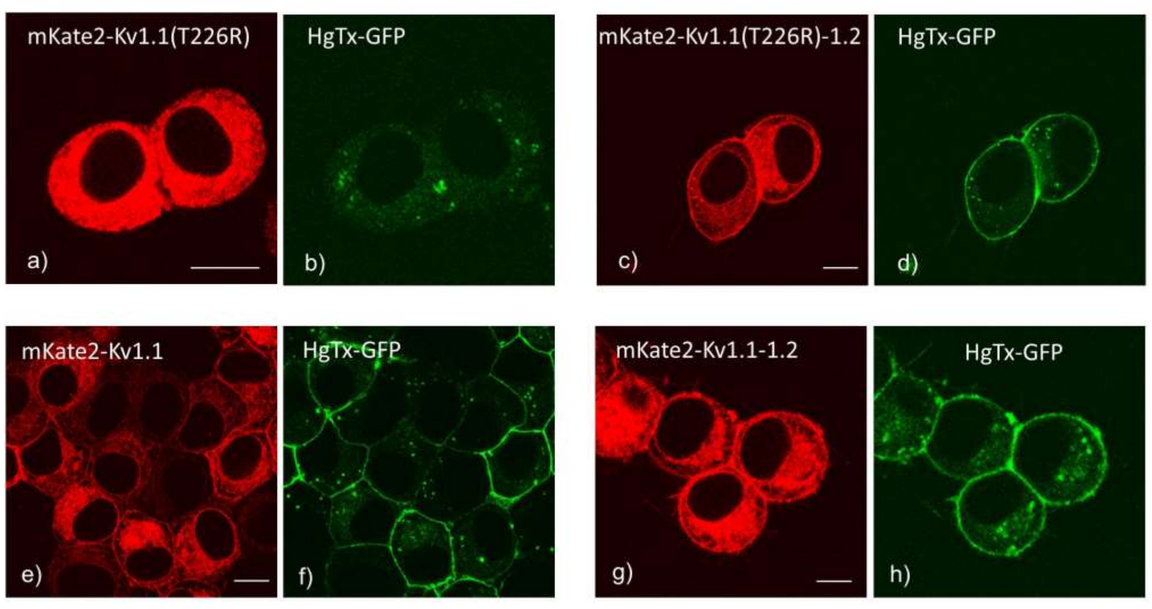

Figure 1. Expression of (a) mKate2-Kv1.1(T226R), (c) mKate2-Kv1.1(T226R)-1.2, (e) mKate2-Kv1.1, (g) mKate2-Kv1.1-1.2 in Neuro2a cells and their interaction with HgTx-G. Cells were incubated with (b,f) 2 nM or (d,h) 0.5 nM HgTx-G for 1 h. Bar is 10 μm.

The binding affinities of HgTx-GFP for the wild type and the mutant heteromers were studied and found to be similar (Fig.2).

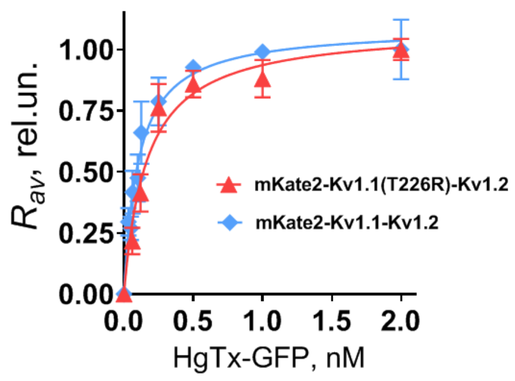

Figure 2. Concentration-dependent binding of HgTx-GFP to heterochannels formed by concatemers mKate2-Kv1.1(T226R)-1.2 and mKate2-Kv1.1-1.2. The R_{av} is the average value of ratio of the fluorescent intensities of the bound ligand to the channels.

Electrophysiological studies show that mKate2-Kv1.1(T226R)-1.2 embedded in plasma membrane do form voltage-gated channels (Fig.3a). The steady-state activation curve of the current transferred by mutant channels was shifted significantly toward more positive membrane potentials compared to that for mKate2-Kv1.1-1.2 channels (Fig.3b). There was no significant difference in the slope of activation curves for the mutant and wild type heterochannels. The mKate2-Kv1.1(T226R)-1.2 channels showed a significantly slower activation and deactivation rates compared to the wild-type heterochannels.

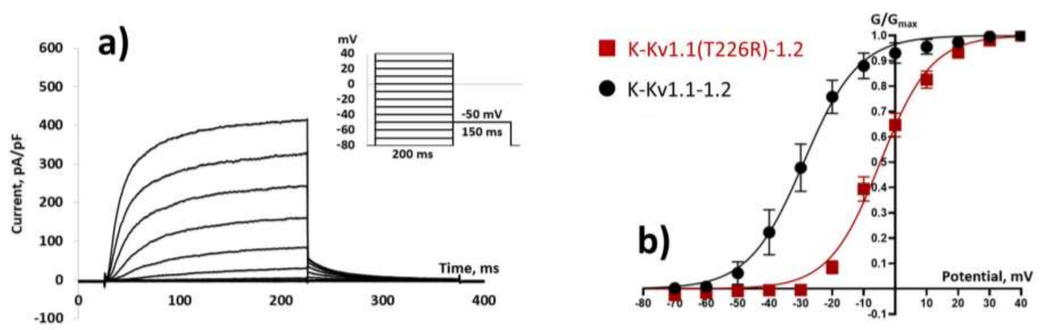

Figure 3. Comparison of potassium currents, transferred by heterochannels mKate2-Kv1.1(T226R)-1.2 and mKate2-Kv1.1- 1.2 in Neuro-2a. a) Representative traces of current transferred by channels mKate2-Kv1.1(T226R)-1.2. The current was induced by square-pulse depolarization protocol shown at the inset. b) Dependences of averaged normalized conductivities (G/Gmax) of cells expressing heterochannels mKate2-Kv1.1(T226R)-1.2 and mKate2-Kv1.1-1.2.

The results obtained provide a clear evidence that T226R mutation disrupts the channel's ability to insert into the cell membrane. However, Kv1.2 subunit used as a partner in the heterochannel mKate2-Kv1.1(T226R)-1.2 improved membrane insertion. Heterochannels mKate2-Kv1.1(T226R)-1.2 exhibited a loss-of-function effect compared to the wt heterochannel mKate2-Kv1.1-1.2 in terms of activation threshold and kinetic constants.

CONCLUSION

The data obtained provide insights into the molecular mechanisms of epilepsy associated with the T226R mutation in the Kv1.1 subunit of the Kv1 channels.

REFERENCES

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