Differential Effects of GRK2 and GRK5 on Arrestin Recruitment Kinetics

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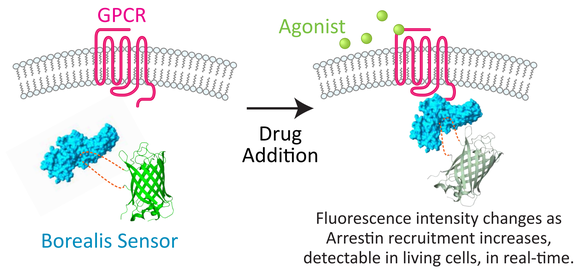
Overview

- Although different GRK isoforms can enhance overall arrestin recruitment to specific GPCRs, the effect of each isoform on the kinetics of arrestin recruitment has not been systematically evaluated.
- Here, we apply a genetically encoded fluorescent biosensor and kinetic data analysis to investigate the effect of GRK2 and GRK5 on arrestin recruitment dynamics.
- For muscarinic and opioid receptors, in most cases GRK2 greatly accelerated recruitment and also induced appreciable complex degradation, whereas GRK5 produced a slower and more sustained arrestin recruitment relative to GRK2.
- Our data suggests that these receptors could be more susceptible to rapid desensitization, internalization and degradation in certain biological systems where GRK2 is highly expressed.

Methods

- GRK isoforms or dominant negative mutants were transiently expressed in HEK293T cells along with GPCRs using BacMam vectors.
- The Borealis arrestin biosensor was used to continuously report arrestin recruitment over time.
- Curve fitting methods for kinetic data were used to extract kinetic parameters such as initial rate, degradation half-time and peak arrestin recruitment as previously described. (Hoare et al., Front. Cell Neurosci. 2022 Jan 17;15:814547).



M1 Muscarinic Acetylcholine Receptor

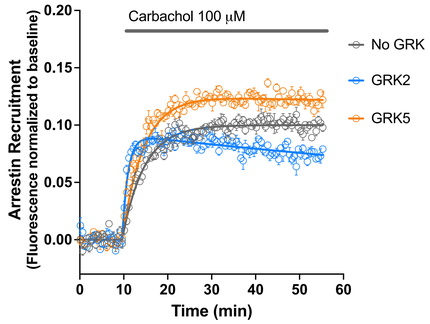


Fig. 1 M1 muscarinic receptor: GRK2 enhanced the initial rate but not the peak arrestin recruitment, and greatly increased the degradation rate (t½ reduced from >100 to 2.5 minutes). GRK5 enhanced peak arrestin recruitment but had a smaller effect on the initial rate and degradation half-time. This indicates that GRK2 promotes fast arrestin recruitment and degradation, while GRK5 promotes a slower and more sustained arrestin signal.

M1	Kinetic Data Analysis		
	Peak Recruitment (NFU)	Initial Rate (NFU/min)	Degradation t½ (h)
No GRK	0.122	0.020	>100
GRK2	0.105	0.069	2.5
GRK5	0.141	0.026	17.0

M3 Muscarinic Acetylcholine Receptor

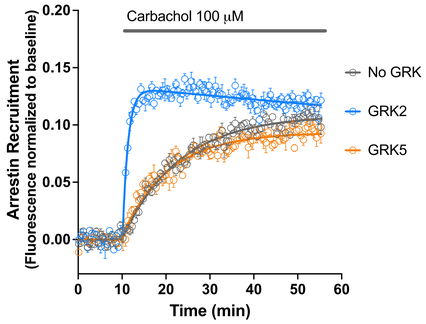


Fig. 2 M3 muscarinic receptor: GRK2 enhanced arrestin recruitment peak and initial rate, as well as degradation, while GRK5 had only a modest effect on the initial rate. The data indicate that GRK2 is responsible for accelerating arrestin recruitment to the M3 receptor.

M3	Kinetic Data Analysis		
	Peak Recruitment (NFU)	Initial Rate (NFU/min)	Degradation t½ (h)
No GRK	0.119	0.008	>100
GRK2	0.151	0.124	4.2
GRK5	0.110	0.009	>100

Kappa Opioid Receptor

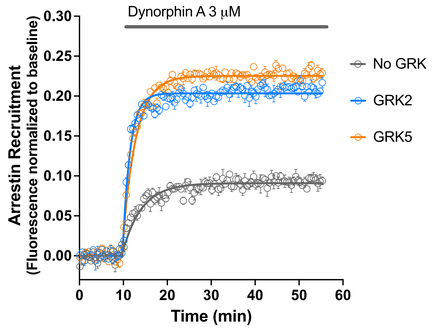


Fig. 3 Kappa Opioid receptor: GRK isoforms enhanced the peak arrestin recruitment and the initial rate, but GRK5 had a smaller effect on the initial rate and a greater effect on the peak arrestin recruitment. These findings suggest that GRK5 promotes a slower and more sustained arrestin recruitment to the Kappa Opioid receptor.

KOR	Kinetic Data Analysis		
	Peak Recruitment (NFU)	Initial Rate (NFU/min)	Degradation t½ (h)
No GRK	0.125	0.025	>100
GRK2	0.241	0.095	>100
GRK5	0.271	0.068	>100

Delta Opioid Receptor

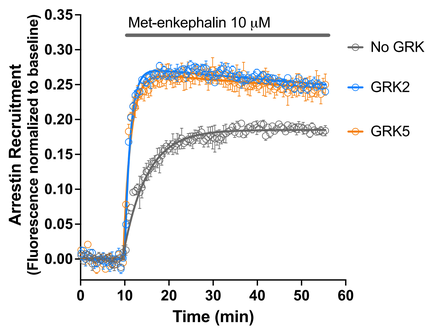


Fig. 4 Delta Opioid receptor: both GRK isoforms enhanced the arrestin recruitment peak and initial rate, but GRK5 had a smaller effect on the initial rate and a slightly smaller effect on the degradation half-time. The data indicates that GRK2 promotes a much more rapid arrestin recruitment, while GRK5 promotes a slower but longer lasting signal.

DOR	Kinetic Data Analysis		
	Peak Recruitment (NFU)	Initial Rate (NFU/min)	Degradation t½ (h)
No GRK	0.205	0.035	>100
GRK2	0.294	0.214	5.5
GRK5	0.295	0.161	5.8

D2 Dopamine Receptor

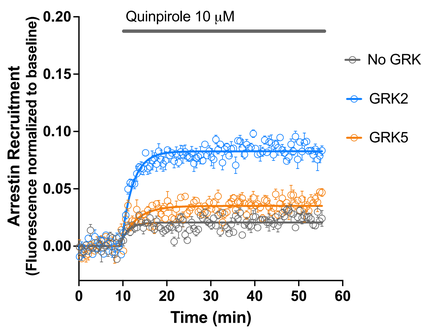


Fig. 5 D2 Dopamine receptor: GRK2 enhanced the peak arrestin recruitment and the intial rate, to a greater extent than GRK5. These results indicate that GRK2 is mostly responsible for arrestin recruitment to the D2 receptor, but GRK5 may also play a role in promoting a sustained arrestin recruitment.

D2	Kinetic Data Analysis		
	Peak Recruitment (NFU)	Initial Rate (NFU/min)	Degradation t½ (h)
No GRK	0.041	0.011	>100
GRK2	0.104	0.034	>100
GRK5	0.058	0.014	>100

Research reported in this poster is funded by NIH grant 5R44GM12539003, and the NIH HEAL Initiative through the National Institute of Drug Abuse of the National Institutes of Health under grant number R44DA050357