Using the waveforms of GPCR signaling dynamics in high throughput drug discovery.

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Overview

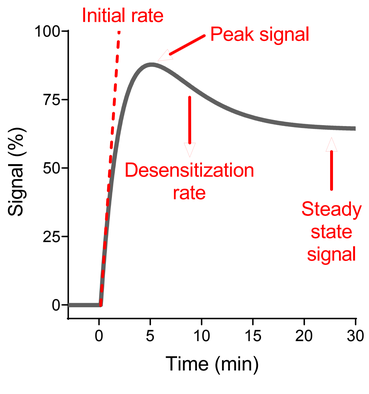
- Signaling dynamics is a new frontier in drug discovery, enabling drugs to signal at the right time and in the right place [1,2].
- Signaling dynamics can be measured routinely in high throughout using biosensors, which enable continuous detection of signaling molecules over time [3,4], and the FDSS/µCell, which enables high frequency simultaneous reads of entire microtiter plates.
- However, for time course data to be useful for drug discovery, drug parameters need to be extracted from the data by curve fitting to drive optimization of new molecules.
- Here we present a simple curve fitting platform for analyzing time course data that operates as a plug-in to the program GraphPad Prism [5-6] and apply it to measure signal generation and desensitization rates for a variety of receptor systems.

References
1) Sci Reports 2020, 10: 12263
2) Trends Pharmacol Sci 2018, 39: 148-157
3) J Biomol Screen 2016, 21: 298-305
4) Assay Guidance Manual 2021, pubmed.ncbi.nlm.nih.gov/34606191/
5) Frontiers Cell Neurosci 2022, 15: 814547

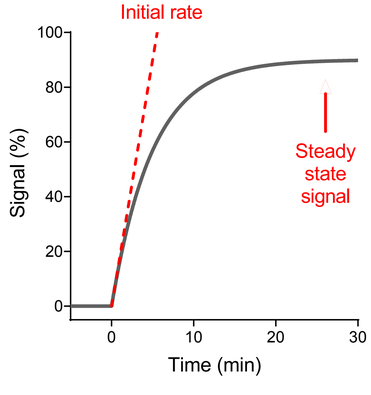
Data Analysis

- Time course equations for a variety of curve shapes entered into GraphPad Prism as a plug-in: www.pharmechanics.com/time-course-tool-pack
- Parameters include:
  - Initial rate of signal generation
  - Fade (desensitization) rate
  - Steady-State signal level
  - Peak level and peak time

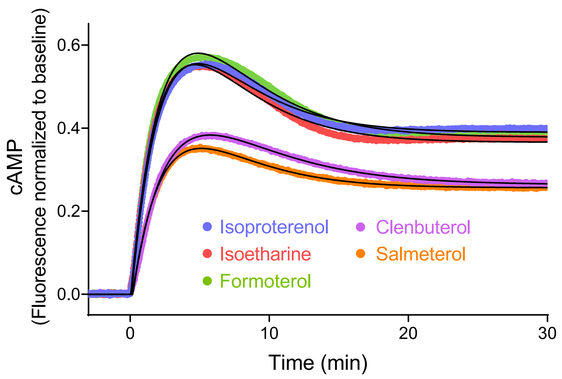
Rise and fall to steady-state curve



Rise to steady-state curve



Signaling dynamics of multiple drug compounds

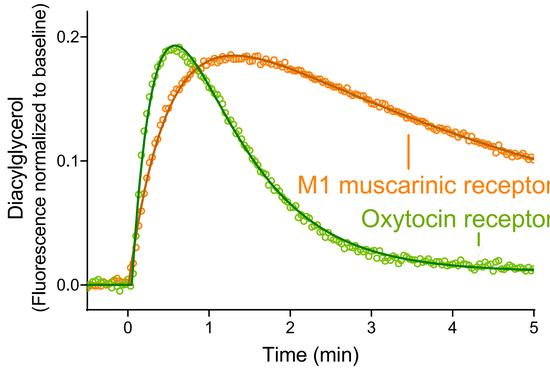


Drug	Initial rate (% isoprot.)	Desensitization $t_{1/2}$ (min)
Isoproterenol	100 [A]	2.2
Isoetharine	102	2.2
Formoterol	101	2.4
Clenbuterol	**60**	**3.5**
Salmeterol	**52**	2.4

A, initial rate for isoproterenol is 0.37 normalized fluorescence units per min

- Different dynamics of cAMP generation by β2 adrenergic receptor agonists.
- Clenbuterol and salbutamol generate cAMP more slowly and clenbuterol desensitizes receptor more slowly.

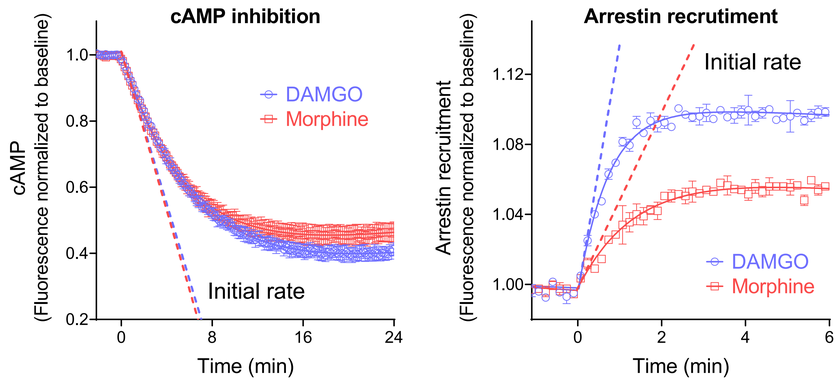
Different signaling by two receptors



	Initial rate (Fl. Units per min)	Desens. $t_{1/2}$ (min)
M1 muscarinic receptor	0.5	3.6
Oxytocin receptor	1.0	0.5

- Faster signal generation and much faster desensitization of oxytocin receptor compared with M1 muscarinic receptor.

Signaling by µ-opioid agonists



Compound	cAMP initial rate (% DAMGO)	Arrestin initial rate (% DAMGO)	cAMP : arrestin initial rate ratio
Peptides			
DAMGO	100	100	1.0
Met-enkephalin	90	97	0.9
Endomorphin-2	96	71	1.4
Small molecules			
Morphine	103	33	3.1
Fentanyl	107	46	2.3
Hydromorphone	94	22	4.3
Buprenorphine	78	0	> 10

- All compounds generate cAMP at a similar rate, except buprenorphine (a partial agonist).
- Small molecules recruit arrestin more slowly than peptides.
- The ratio of initial rates can be used to quantify bias for cAMP over arrestin – small molecules are biased for cAMP.

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