

Pharmacology of Purinoceptors:
Comparison of the Effects of Agonists on
Membrane Potential and Ca^{++}_i

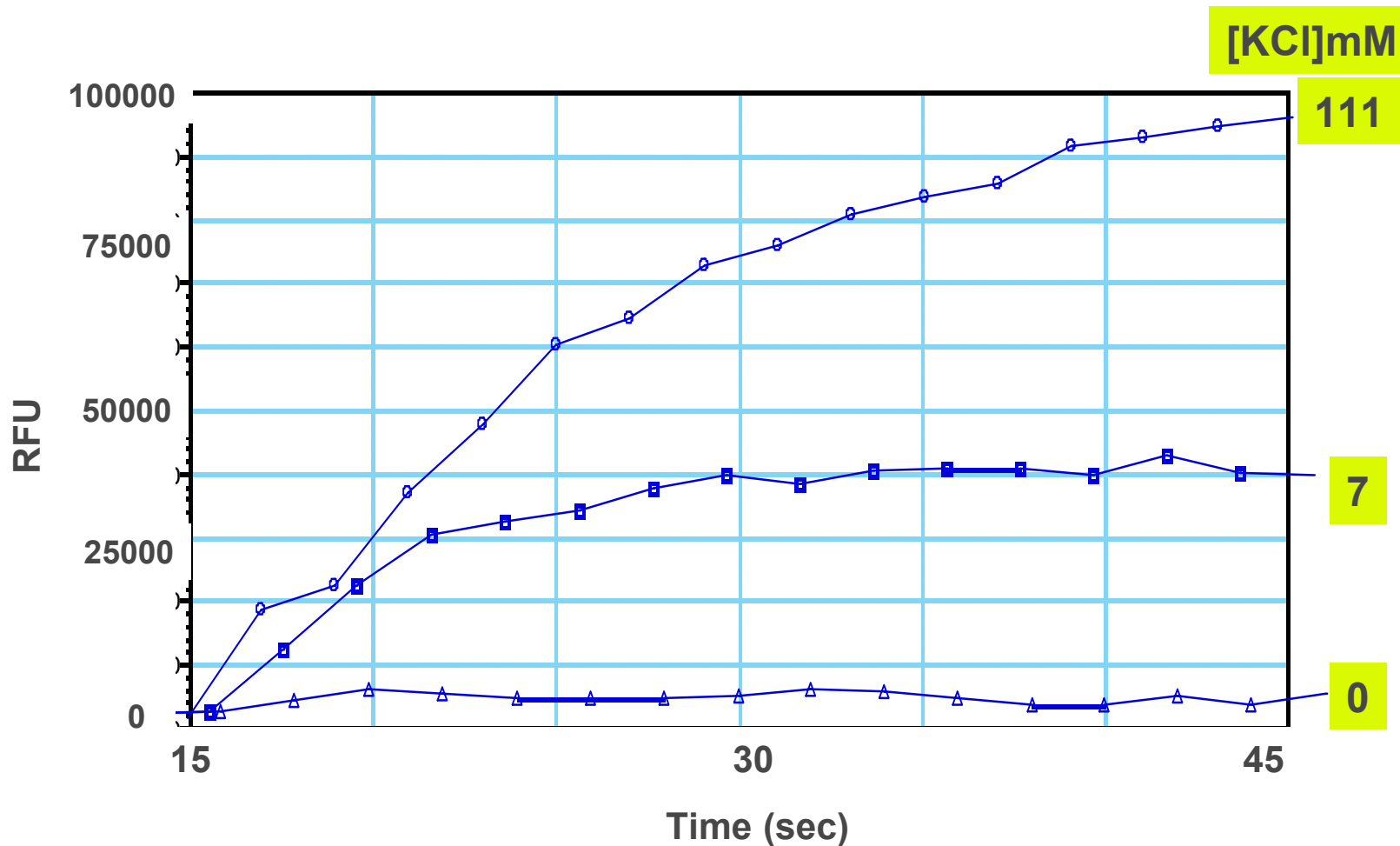
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Fifth Annual Cell Analysis Products Users Meeting

FLEXstation:

- ◆ A fluorescence plate reader
- ◆ 96-well format
- ◆ Able to excite and to read emissions at variable wavelengths
- ◆ Fluidics capabilities -- can perform up to three additions

KCl-mediated increase in fluorescence with membrane potential dye



Effects of ATP and UTP

- ◆ Investigated the effects of purinoceptor agonists on an endogenous purinoceptor in CHO.K1 cells loaded with:
 - a) Fluo-3
 - b) MDC membrane potential assay kit

Purinoreceptor family:

- ◆ P2X -- ligand gated ion channels
- ◆ P2Y -- G-protein coupled receptors

Couple to Gq → → Increase Ca^{++}_i via activation of PLC

P2Y agonist potencies:

◆ P2Y₁ ADP > ATP

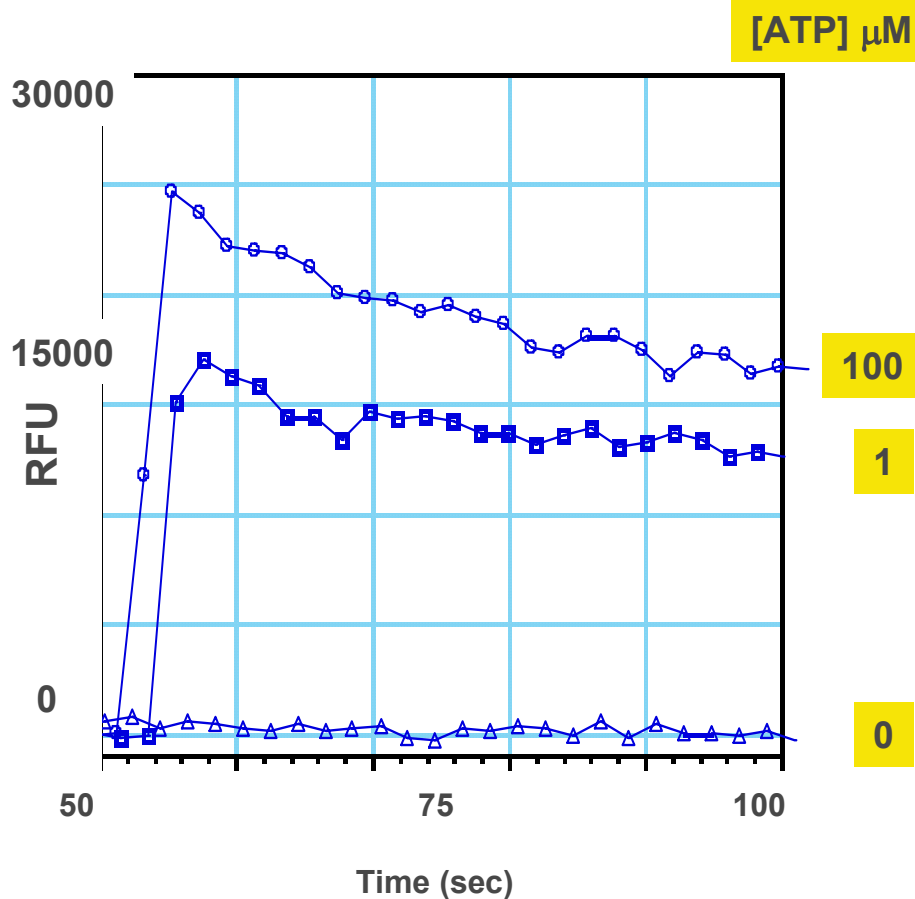
◆ P2Y₂ UTP \simeq ATP

◆ P2Y₄ UTP > ATP

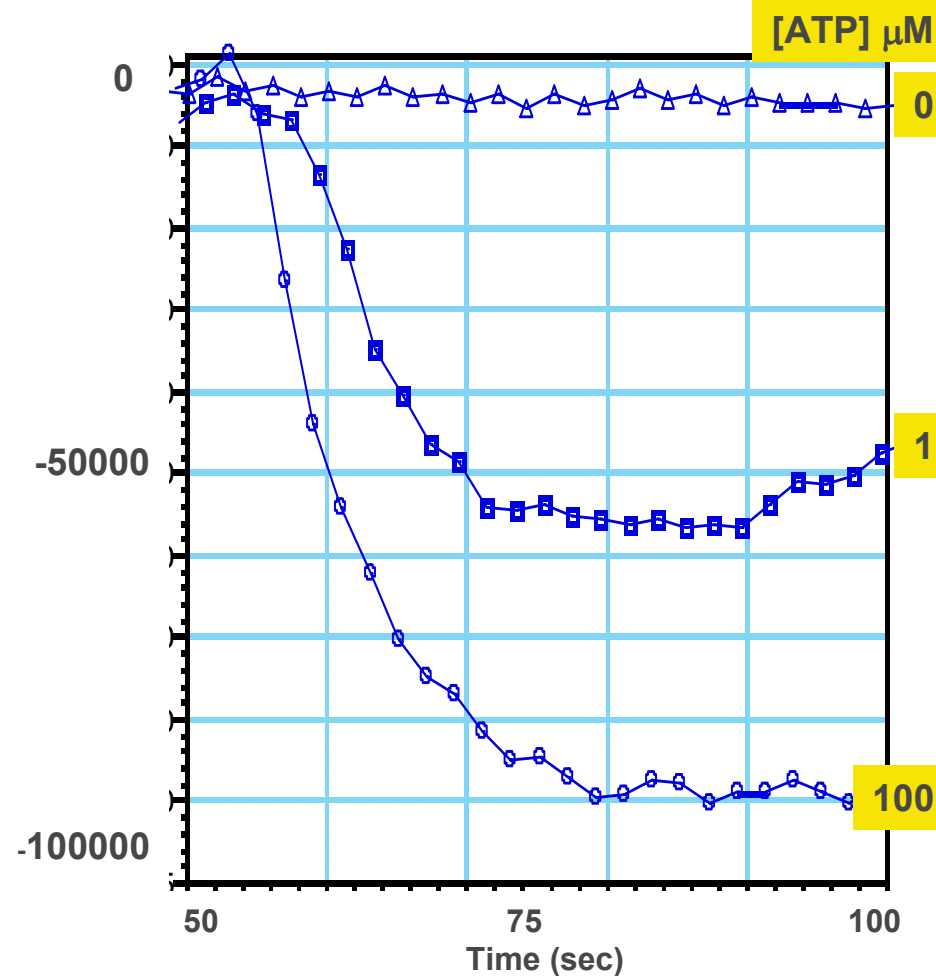
◆ P2Y₆ UDP >> UTP >> ATP

Response to ATP in CHO.K1

Raw Data



Fluo-3



Membrane Potential Dye

Purinoreceptor-mediated responses using Fluo-3 or membrane potential dye

- ◆ UTP or ATP concentration-dependently increase fluorescence in cells loaded with Fluo-3
- ◆ UTP or ATP concentration-dependently decrease fluorescence in cells loaded with membrane potential dye

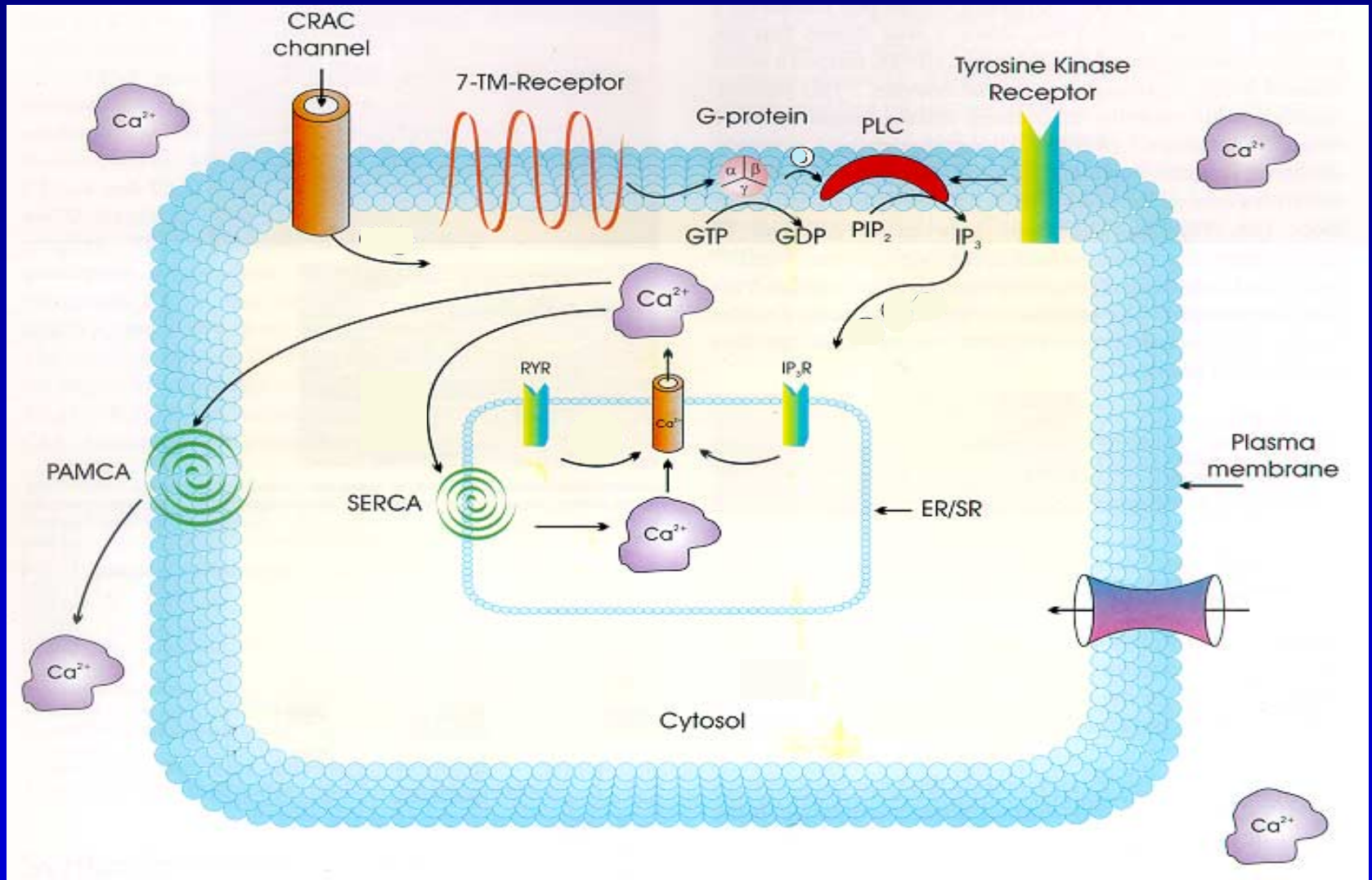
pEC₅₀ values

Fluo-3 vs. membrane potential dye

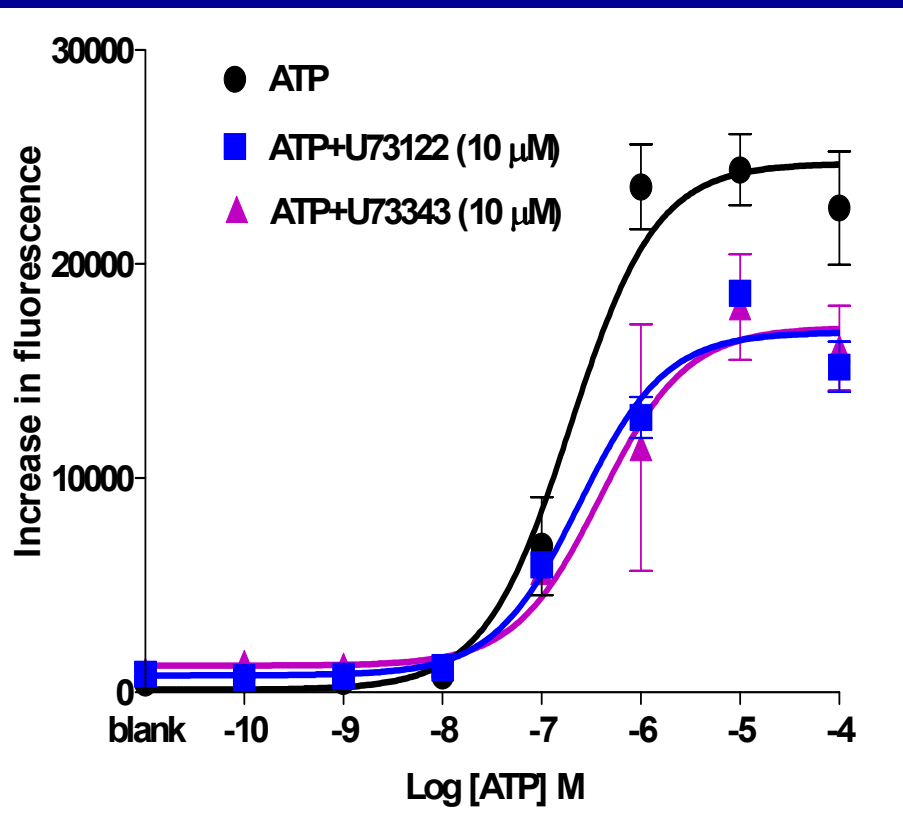
	pEC50 values (log M)	
	<u>Fluo-3</u>	<u>Membrane Potential Dye</u>
ATP	6.8 \pm 0.2	6.4 \pm 0.1
UTP	6.7 \pm 0.2	6.4 \pm 0.2

- P2Y₂ mediated effect (ATP = UTP)
- Fluo 3 pEC₅₀ \approx membrane potential pEC₅₀

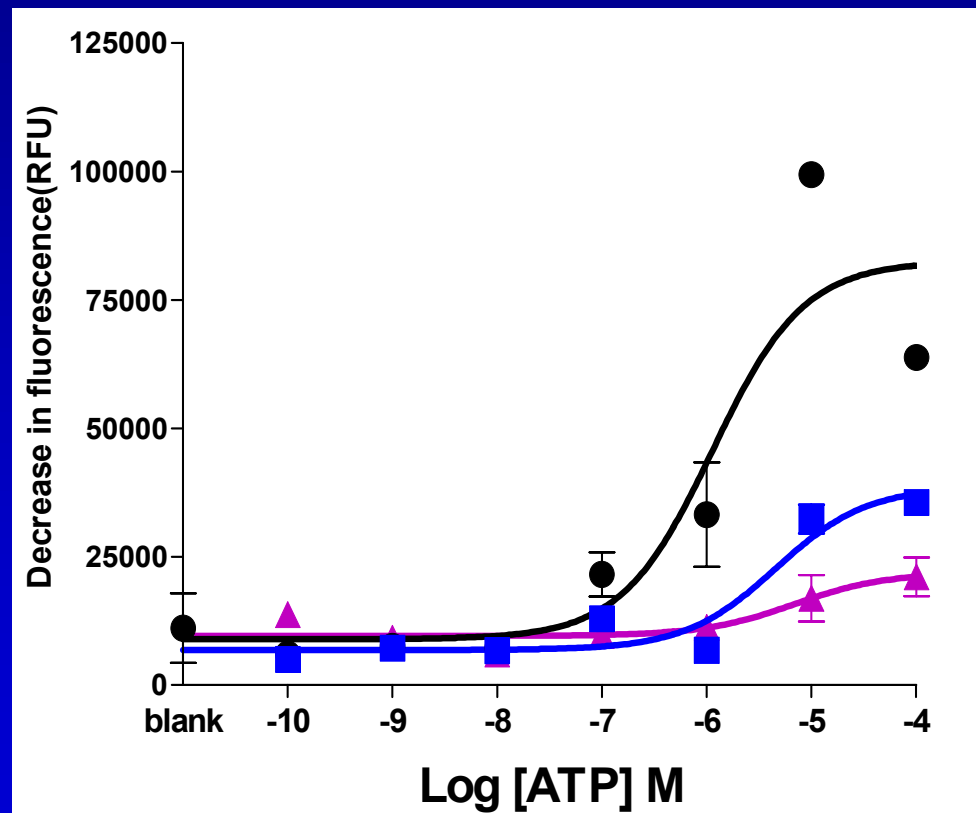
Intracellular Ca^{2+} Regulation



Responses to ATP in CHO.K1 effects of PLC Inhibitors U73122 or U73343

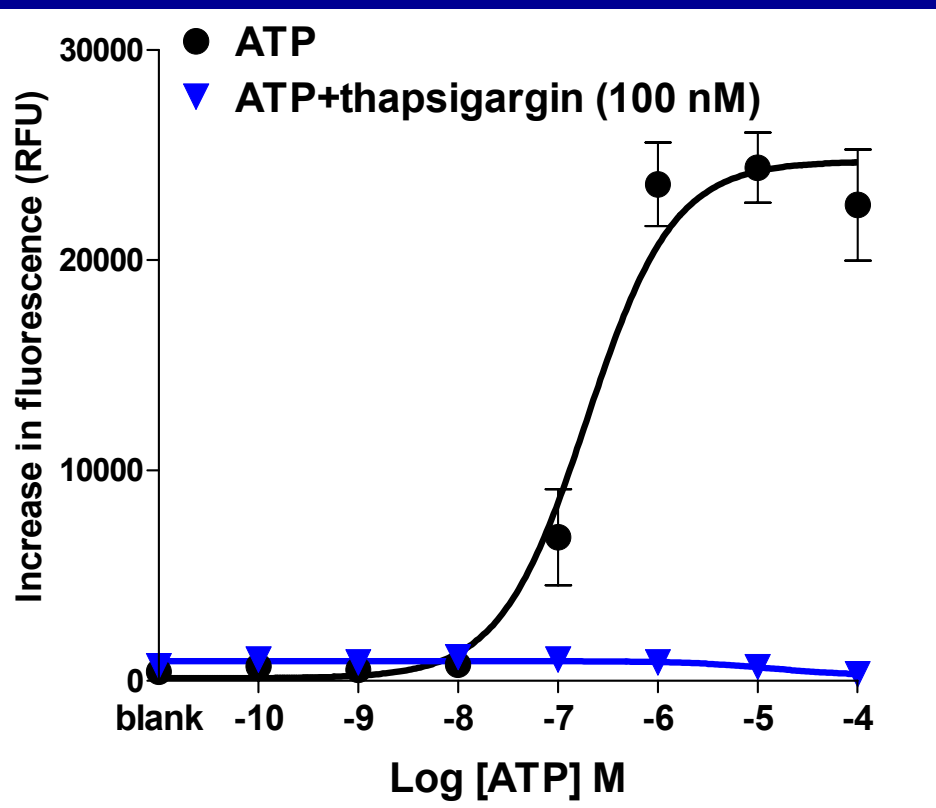


Fluo-3

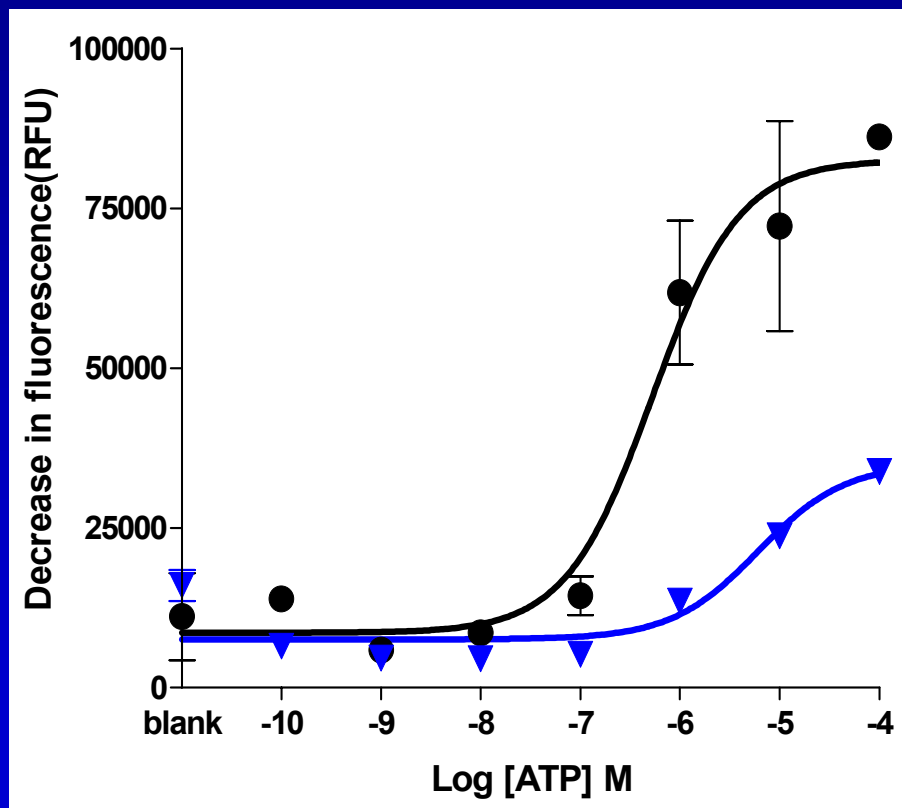


Membrane Potential Dye

Responses to ATP in CHO.K1 effect of Thapsigargin



Fluo-3



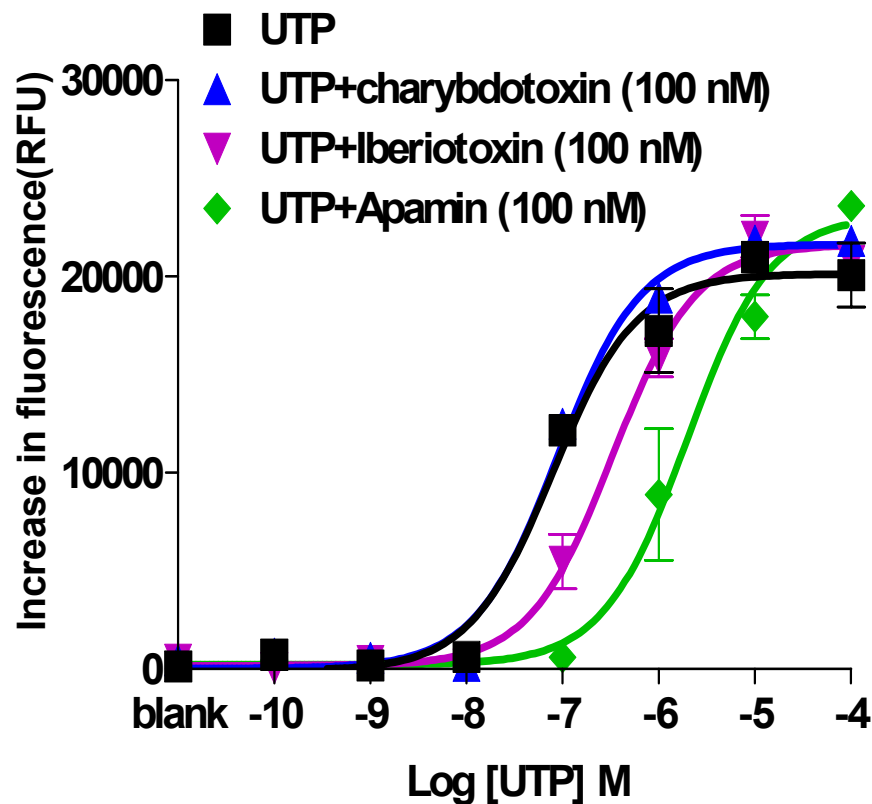
Membrane Potential Dye

Possible mechanism of CHO.K1 hyperpolarization

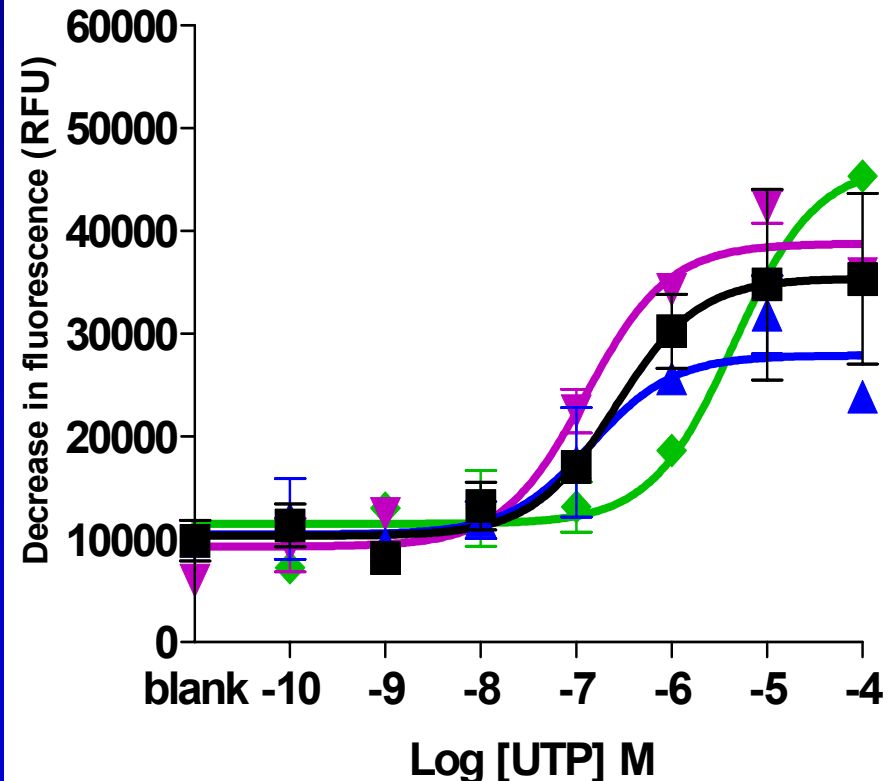
- ◆ **Ca⁺⁺-activated K⁺ channels**
 - sensitive to Apamin, Iberiotoxin, Charybdotoxin

Responses to UTP in CHO.K1

effects of Ca^{++} sensitive K^{+} channel blockers



Fluo-3



Membrane Potential Dye

Conclusions: P2Y₂

- ◆ In CHO.K1 cells Purinoceptor agonists:
 - a) increase Ca^{++}_i concentration
 - b) hyperpolarize cells
- ◆ Agonist potencies consistent with P2Y₂ interaction
- ◆ Agonist potencies for Ca^{++}_i response \approx hyperpolarization (pEC₅₀ = 6.8 and 6.4 respectively for ATP)

Conclusions: Ca^{++} and K^+

- ◆ U73122 and U73343 attenuated effects of UTP and ATP
→ involvement of PLC
- ◆ Thapsigargin significantly attenuated effects of UTP and ATP
→ involvement of intracellular Ca^{++} stores
- ◆ Apamin attenuated effects of UTP
→ involvement of small conductance Ca^{++} -sensitive K^+ channels
- ◆ Iberiotoxin and charybdotoxin had no effect on UTP-mediated effects

Future studies

- ◆ antagonist sensitivity – confirm P2Y₂ interaction
- ◆ investigate the selectivity of apamin, charybdotoxin and iberiotoxin for Ca⁺⁺-sensitive K⁺ channel subtypes
- ◆ changes due to
 - voltage sensitive Ca⁺⁺ channels
 - Ca⁺⁺ responsive Ca⁺⁺ channels

Acknowledgements

Renee Martin, Pharmacology and Cell Sciences

Jeannie David, Neurophysiology

Don Button, Pharmacology and Cell Sciences

Joel Gever, Biochemistry

Anne Ferguson, Molecular Devices