

TARGACEPT



200 East First Street, Suite 300
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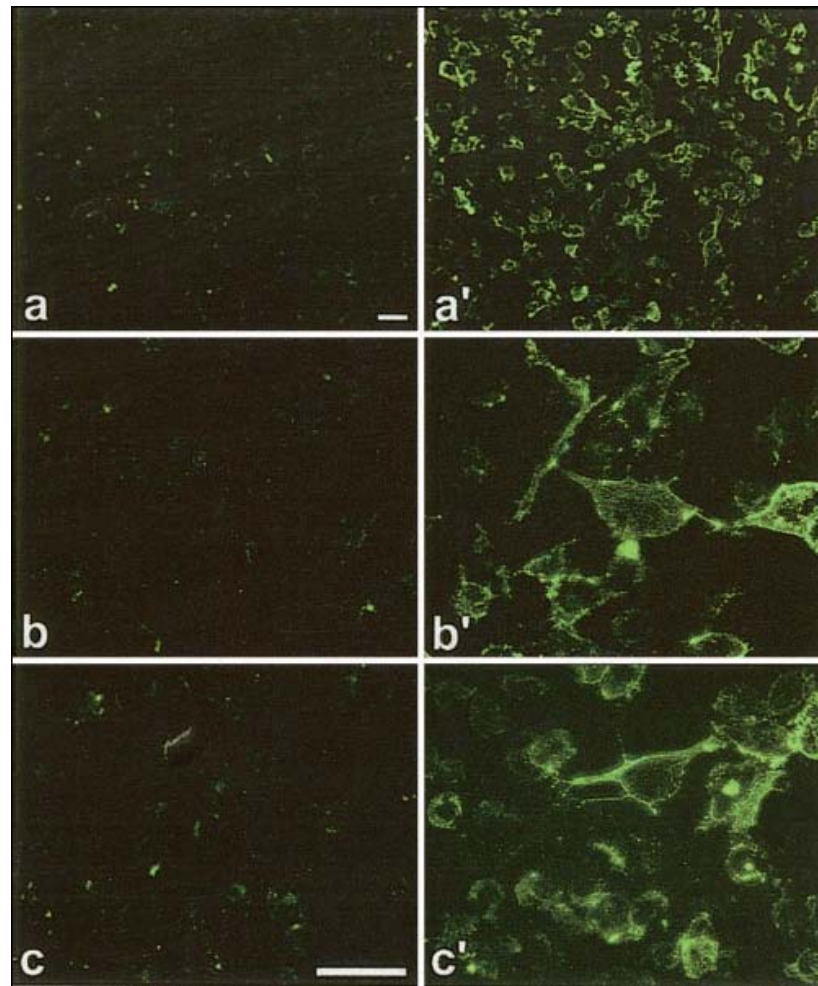
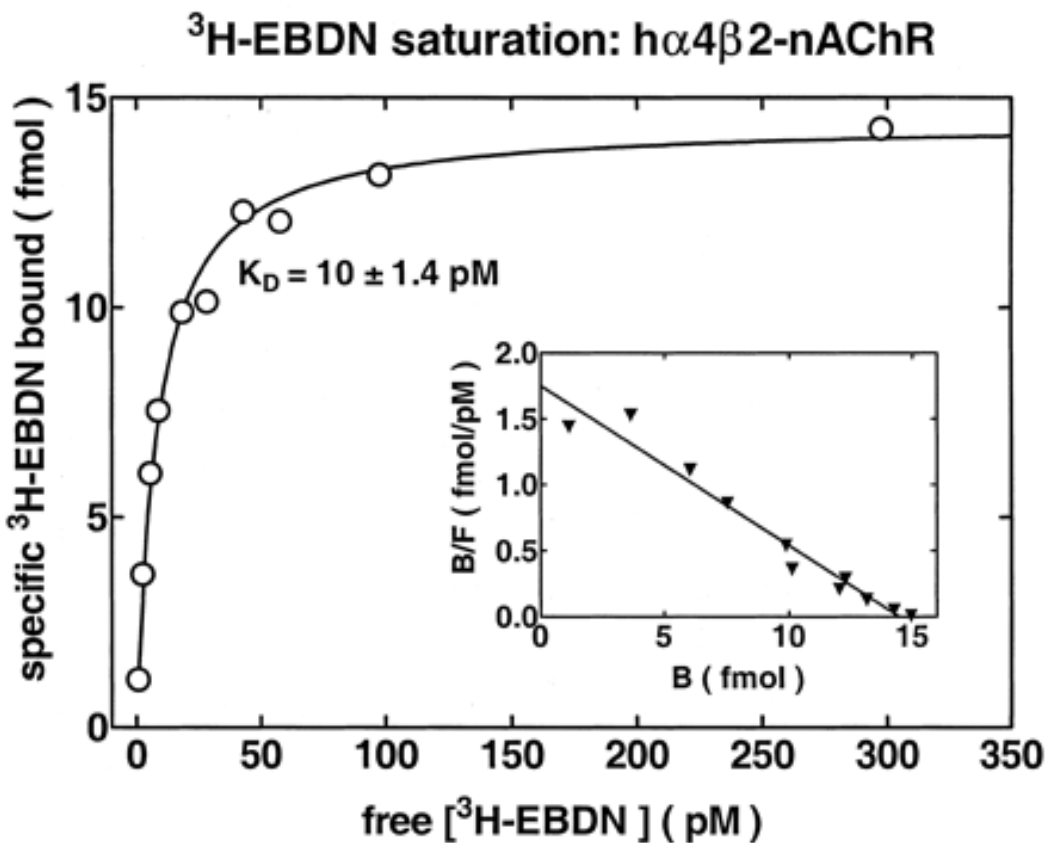
Optimization of Calcium Flux Mediated by
the $\alpha 4\beta 2$ Neuronal Nicotinic Receptors
Utilizing the FLIPR Calcium 4 Assay Kit

Jiahui Zhang, Terry Hauser, Merouane Bencherif

Neuronal Nicotinic Receptors (NNR)

- Cys-loop superfamily; pentameric ligand-gated ion channels
- 12 subunits: α 2-10; β 2-4
- 2 categories
 - high affinity (nM): α 4 β 2
 - lower affinity (μ M): α 7
- Location:
 - Extrasynaptic
 - somatodendritic regions
 - presynaptic terminals
- Mechanisms
 - modulatory
 - neurotransmitter release
 - Na⁺ and Ca²⁺ fluxes associated

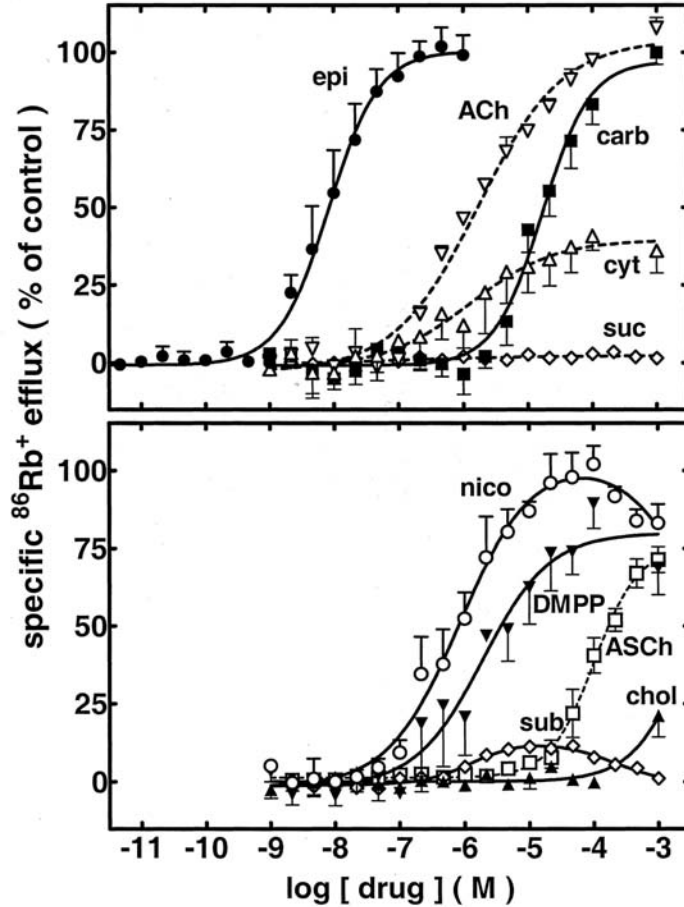
SH-EP1 α 4 β 2 Cells



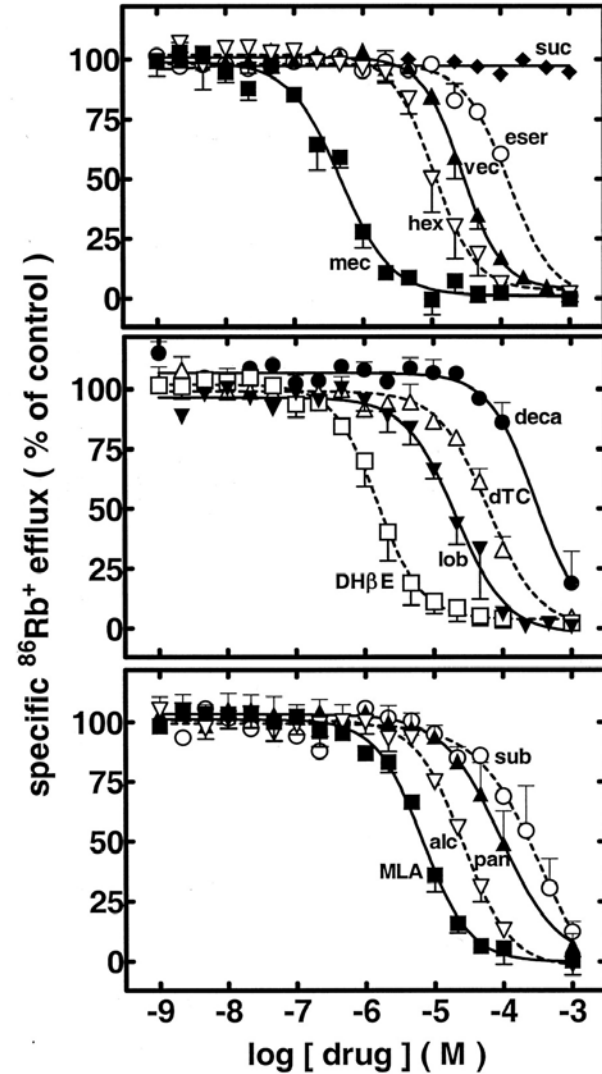
Eaton et al., *Mol Pharmacol* 64:1283, 2003

SH-EP1 α 4 β 2 Cells

Agonist Dose-Response: h α 4 β 2-nAChR



Antagonist Dose-Response: h α 4 β 2-nAChR

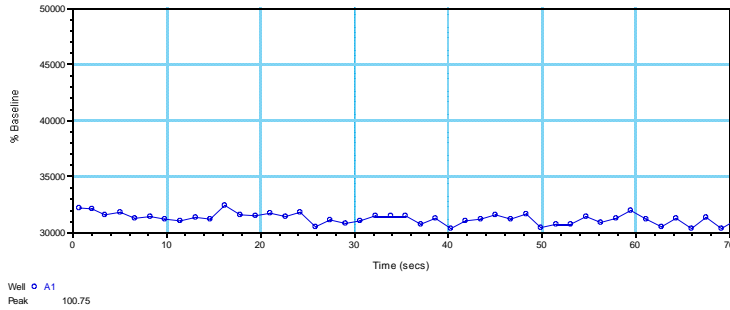


Eaton et al., *Mol Pharmacol* 64:1283, 2003

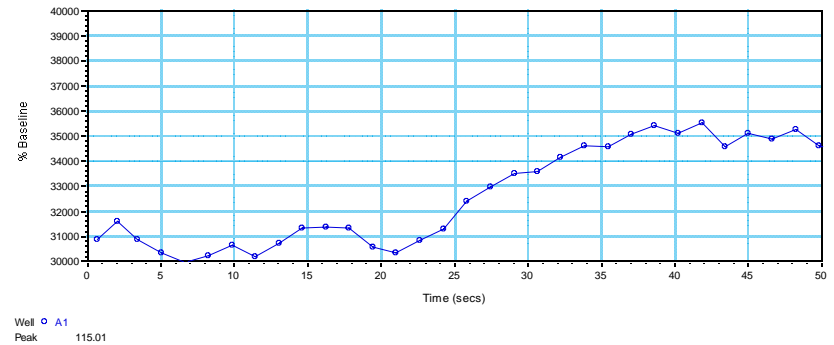
FLIPR Calcium 3 Assay



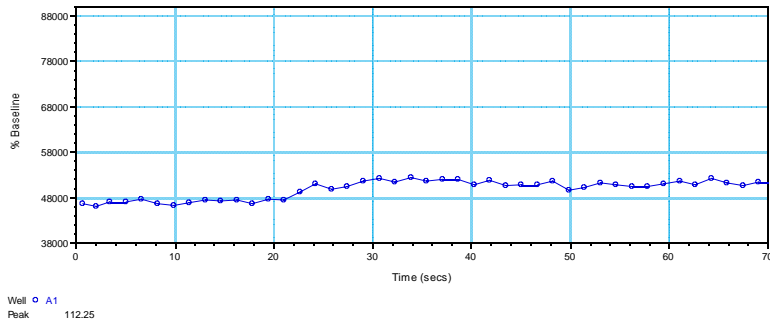
Ca 3, 100 μ M Nic



Fluo 4, 100 μ M Nic



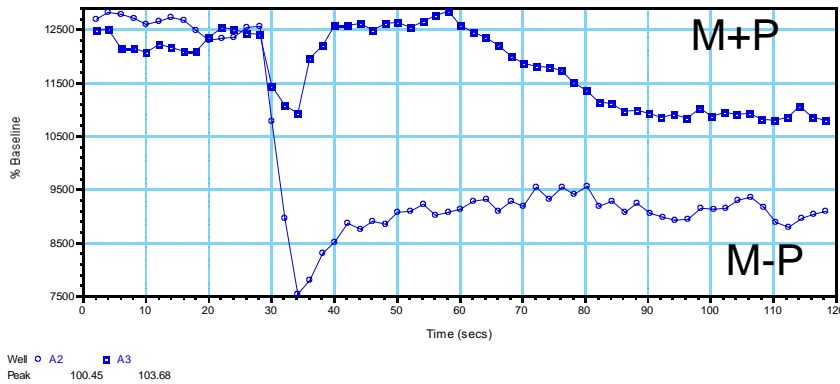
Ca Green, 100 μ M Nic



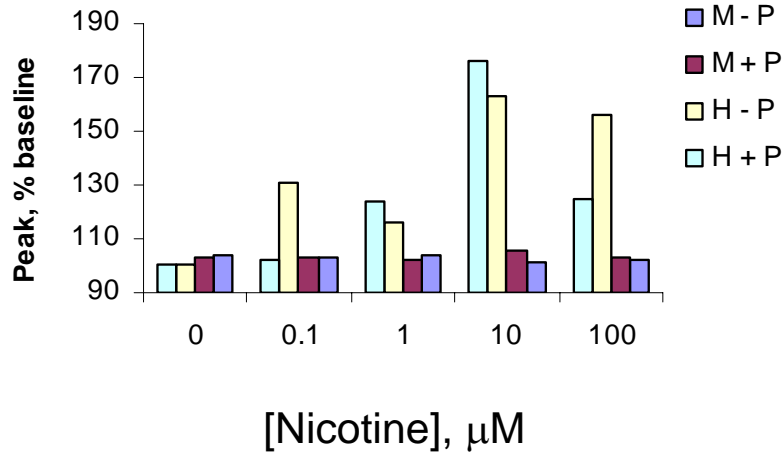
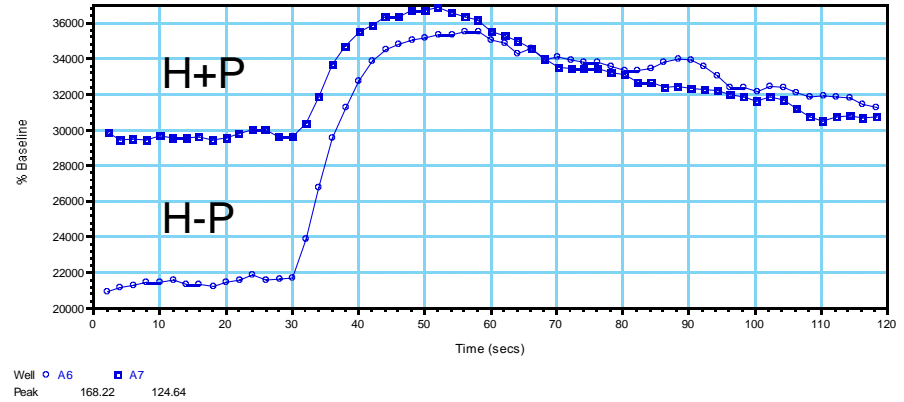
Nic: nicotine

Effect of Media, HBSS/HEPES and Probenicid

100 μ M Nic



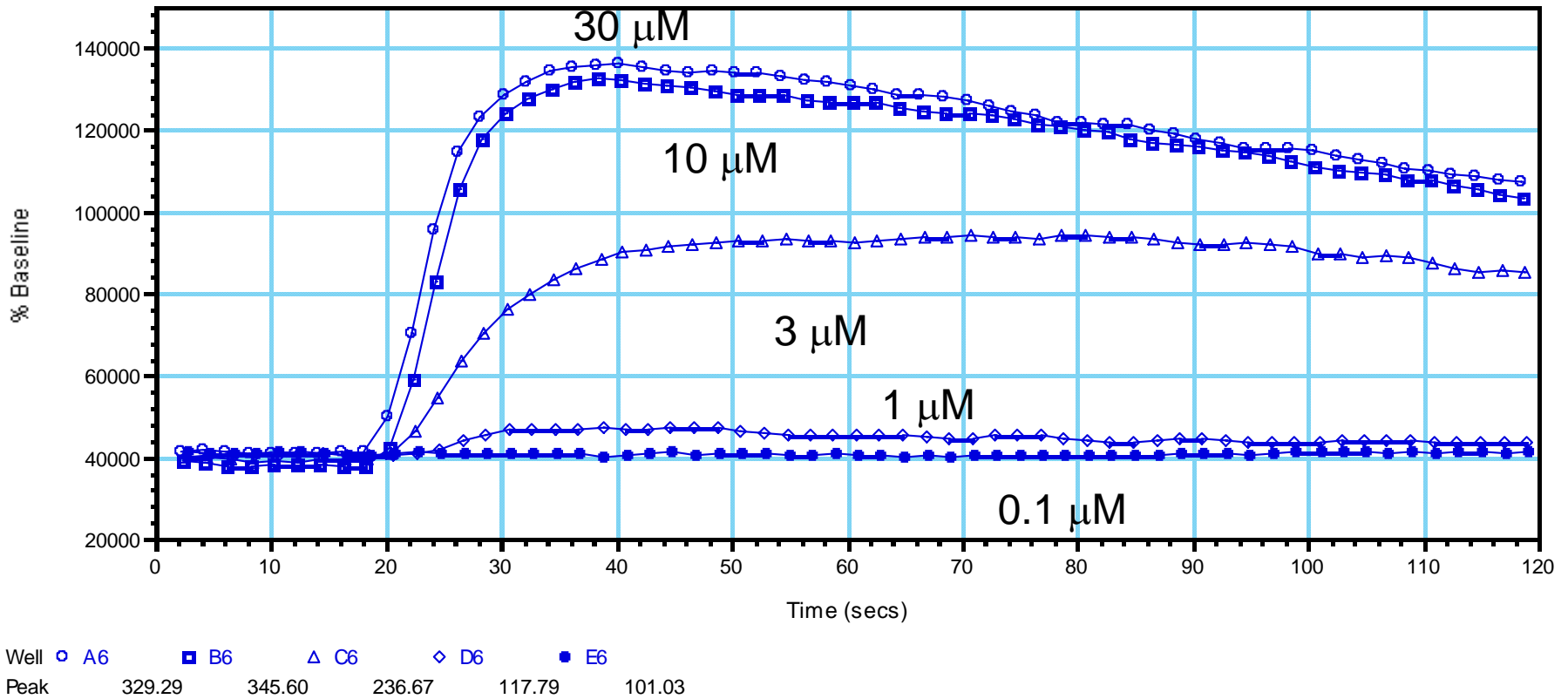
100 μ M Nic



M: media (DMEM + 5% FBS, 10% HS)
H: HBSS/HEPES
P: probenicid (2.5mM)

Effect of MEBSS

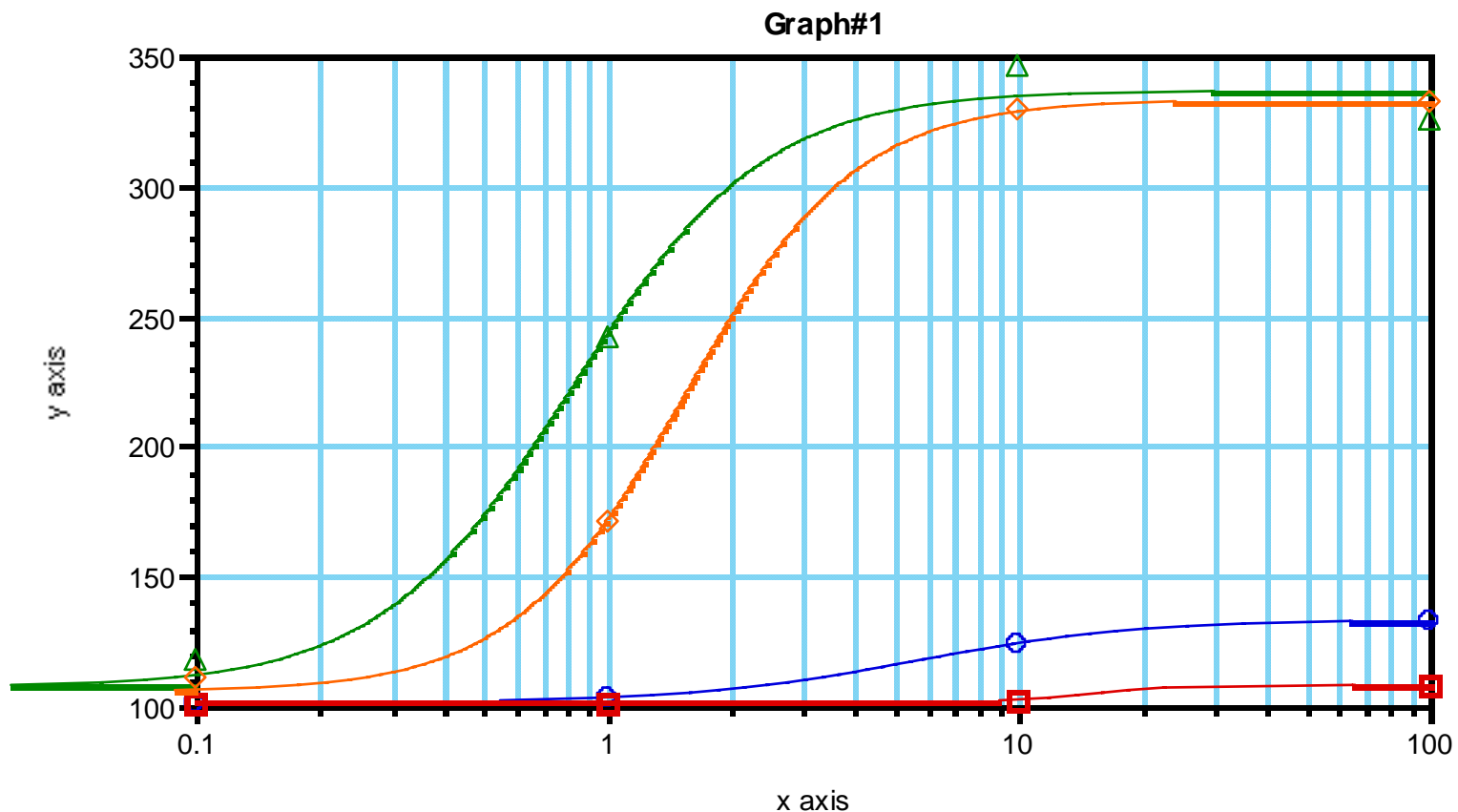
Nicotine response in Modified Earle's Balanced Salt Solution



Buffer Components

Components, mM	MEBSS	HBSS/HEPES
Calcium Chloride (CaCl ₂)	4	1.26
D-Glucose (Dextrose)	5	5.56
HEPES	20	20
Magnesium Chloride (MgCl ₂)		0.49
Magnesium Sulfate (MgSO ₄)	0.8	0.41
N-methyl D-glucamine	120	
Potassium Chloride (KCl)	5	5.33
Potassium Phosphate monobasic (KH ₂ PO ₄)		0.44
Sodium Chloride (NaCl)	20	137.93
Sodium Phosphate dibasic (Na ₂ HPO ₄ ·7H ₂ O)		0.36
TRIS	7	

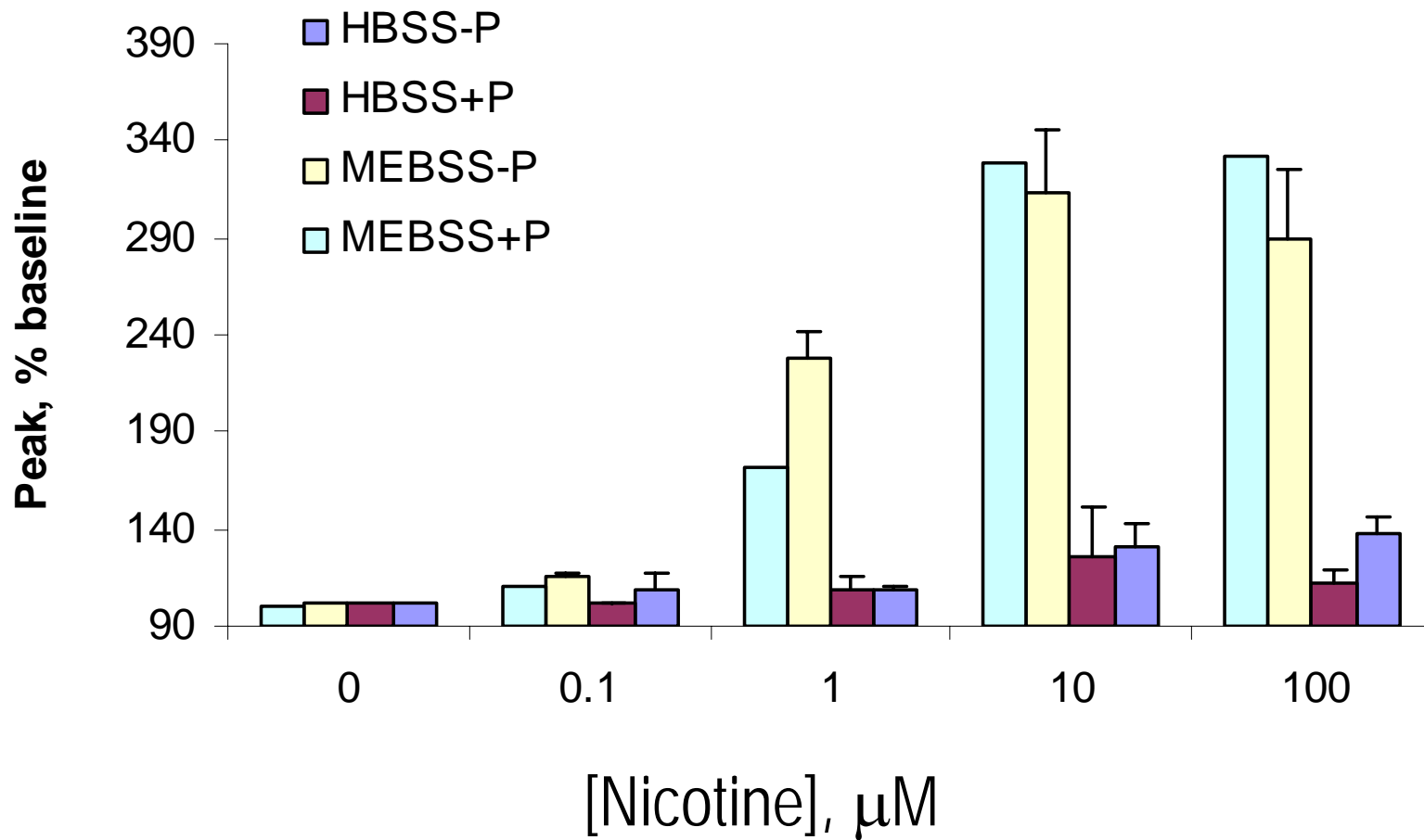
Nicotine concentration-response



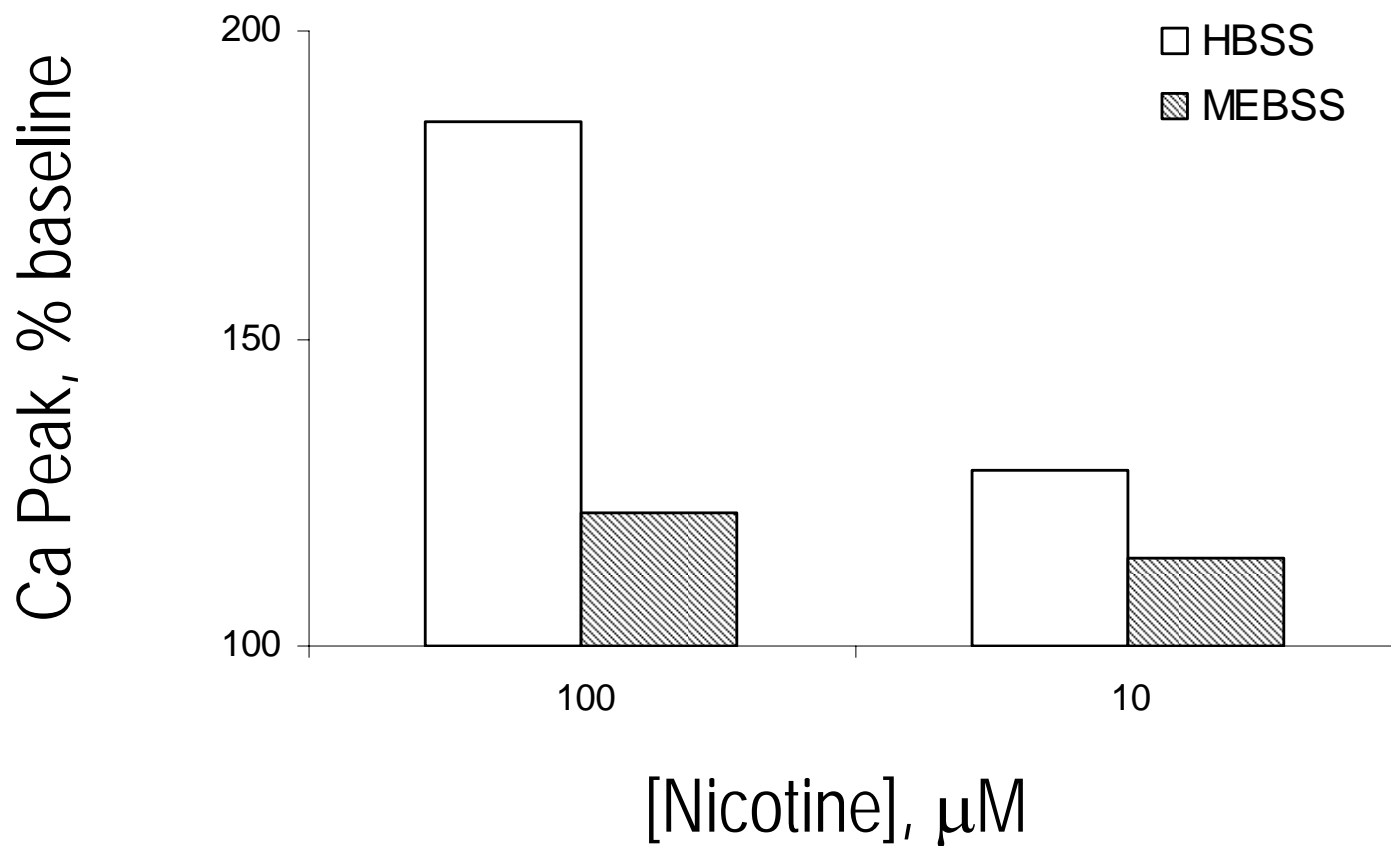
$$y = ((A - D) / (1 + (x/C)^B)) + D$$

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>R²</u>
○ Plot#1 (Nic/HBSS-P: Concentration vs MeanValue)	101.202	1.594	5.633	133.135	0.999
■ Plot#2 (Nic/HBSS+P: Concentration vs MeanValue)	101.384	4.379	15.335	107.886	0.992
△ Plot#3 (Nic/MEBSS-P: Concentration vs MeanValue)	107.205	1.857	0.819	336.517	0.993
◇ Plot#4 (Nic/MEBSS+P: Concentration vs MeanValue)	105.315	2.076	1.542	333.05	0.999

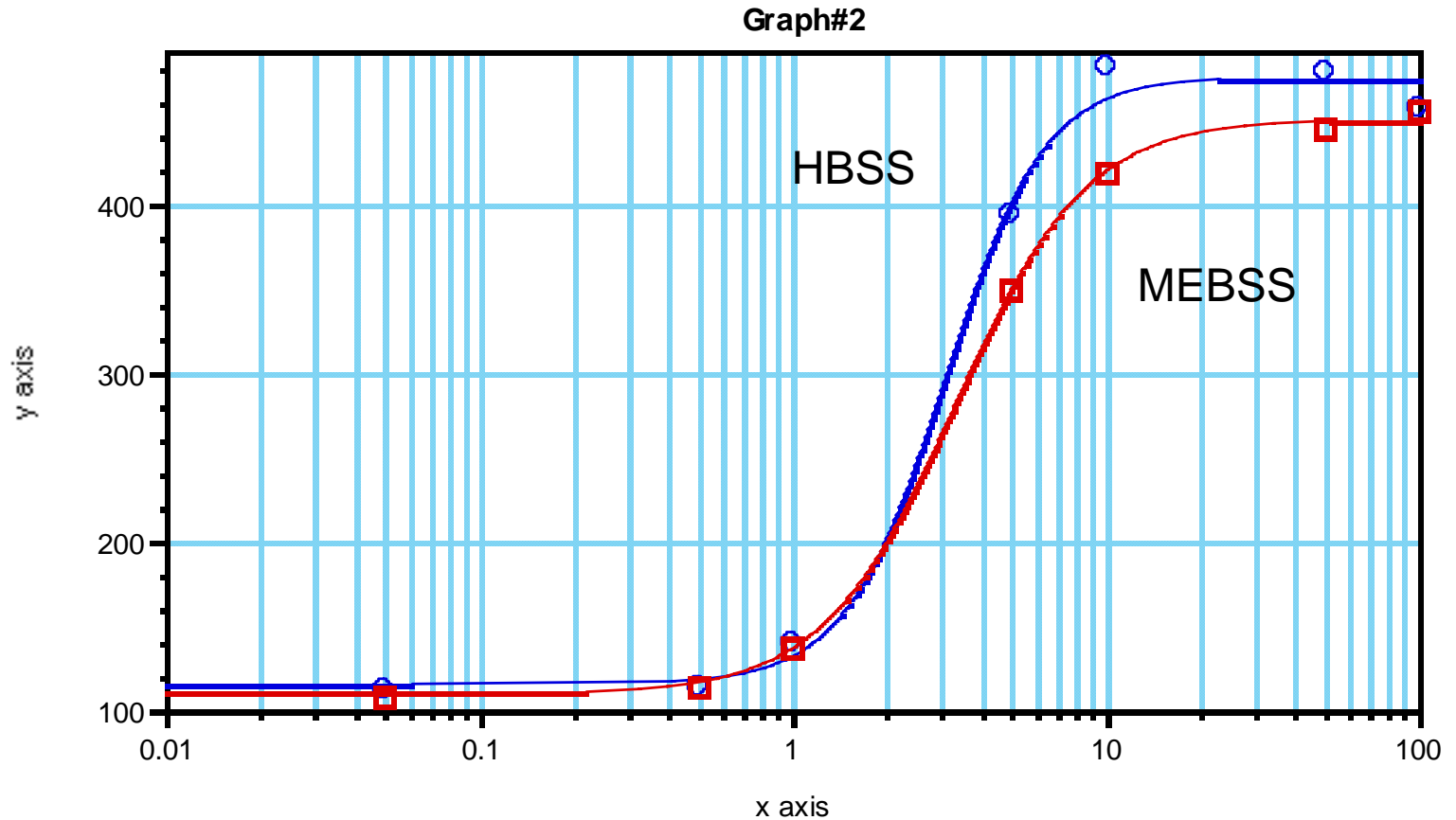
MEBSS vs HBSS +/- P



Nicotine response in HEK $\alpha 4\beta 2$



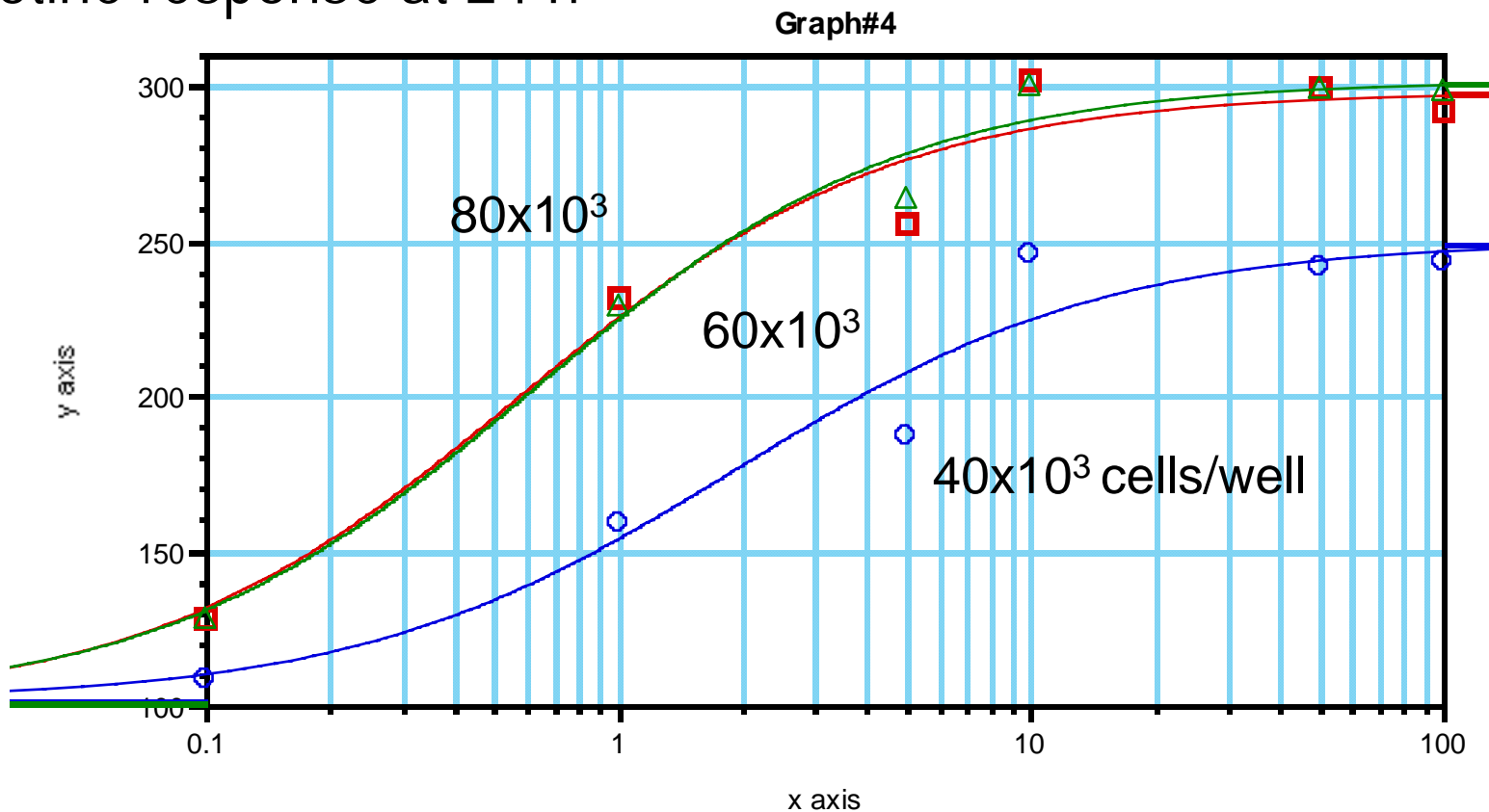
Nicotine response in HEK α 7RIC3



$$y = \left(\frac{A - D}{1 + (x/C)^B} \right) + D$$

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>R²</u>
Plot#1 (Nic, HBSS: Concentration vs MeanValue)	115.346	2.726	3.062	474.606	0.996
Plot#2 (Nic, MEBSS: Concentration vs MeanValue)	109.475	2.06	3.296	449.7	1

Nicotine response at 24 h



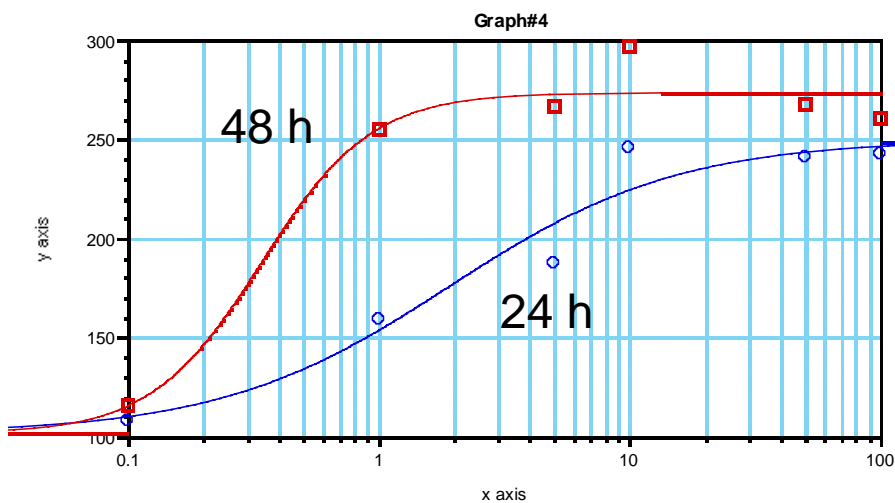
$y = ((A - D) / (1 + (x/C)^B)) + D$:

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>R²</u>
○ Plot#1 (Nic/40/24: Concentration vs MeanValue)	101.346	0.953	1.911	249.579	0.961
■ Plot#2 (Nic/60/24: Concentration vs MeanValue)	99.609	0.955	0.571	297.933	0.982
△ Plot#3 (Nic/80/24: Concentration vs MeanValue)	100.672	0.961	0.615	301.414	0.991

Nicotine response

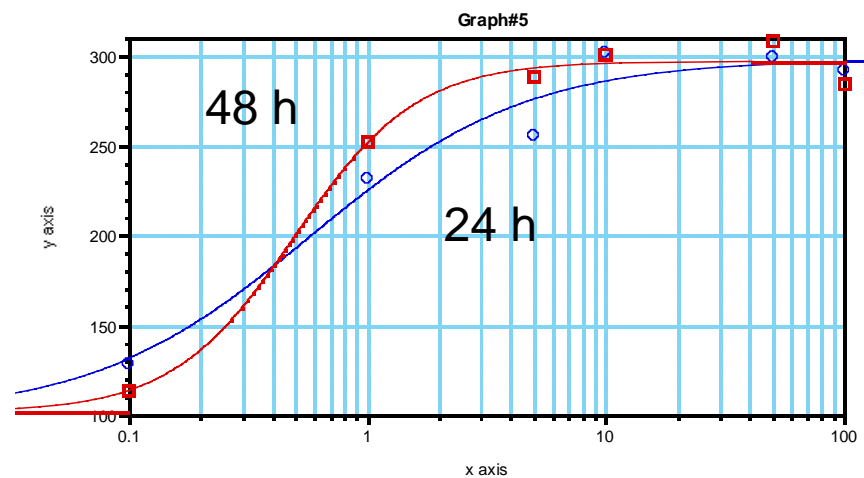
40x10³ cells/well

60x10³ cells/well



$$y = ((A - D)/(1 + (x/C)^B)) + D$$

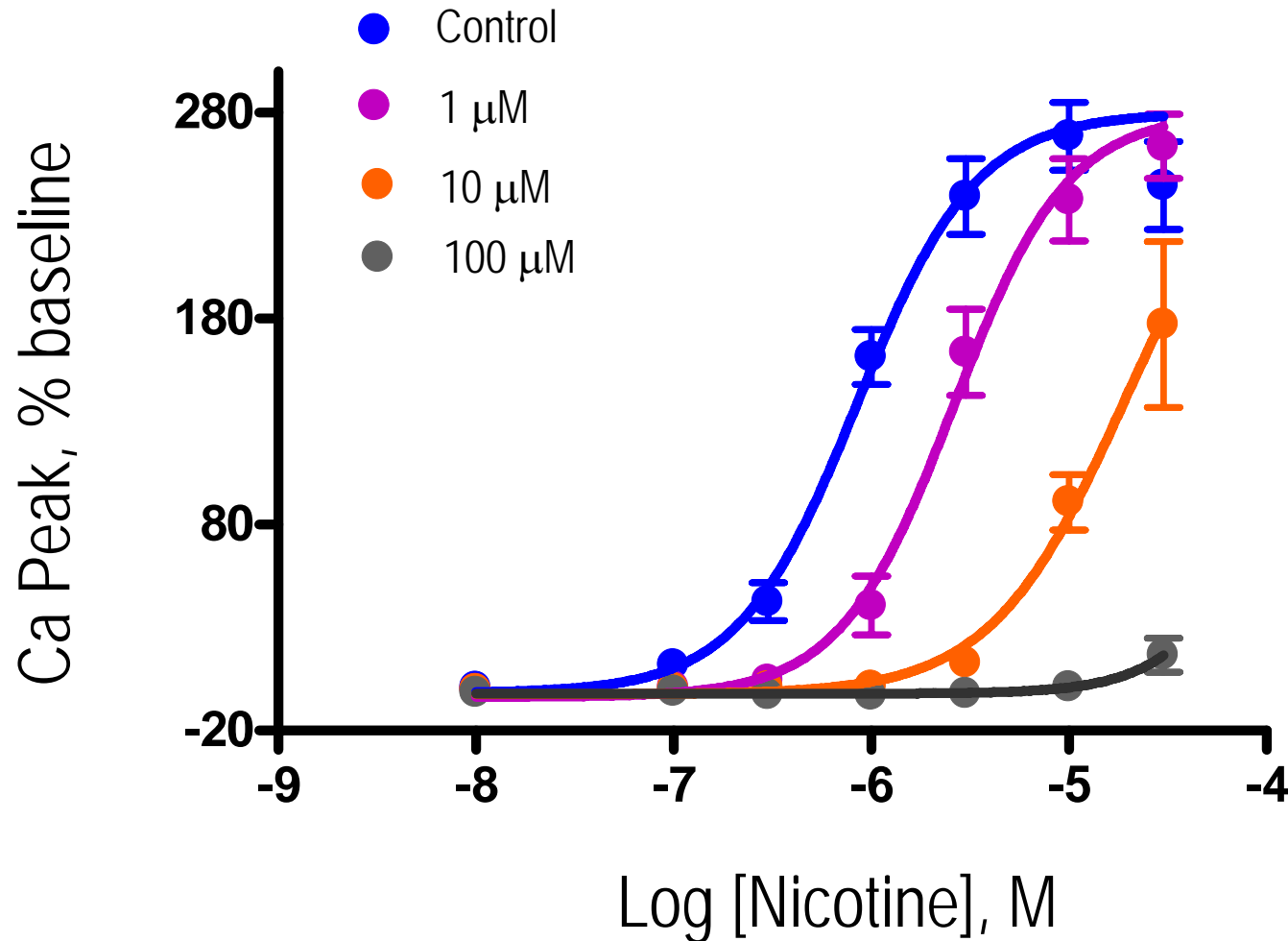
	A	B	C	D	R ²
Plot#1 (Nic/40/24@Plate 1: Concentration vs MeanV...	101.346	0.953	1.911	249.579	0.961
Plot#2 (Nic/40/48: Concentration vs MeanValue)	101.302	1.979	0.341	273.348	0.98



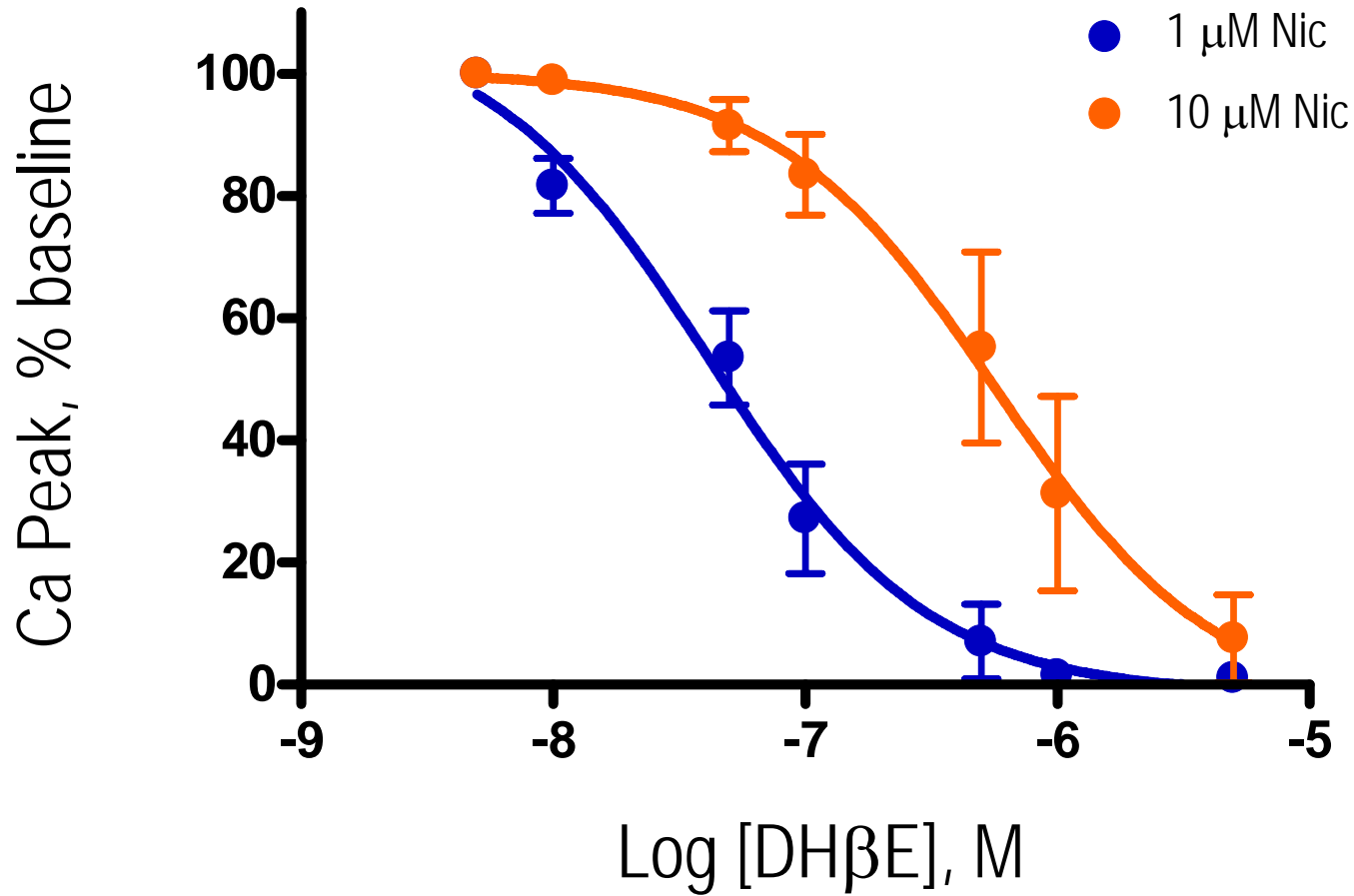
$$y = ((A - D)/(1 + (x/C)^B)) + D$$

	A	B	C	D	R ²
Plot#1 (Nic/60/24@Plate 1: Concentration vs MeanV...	99.609	0.955	0.571	297.933	0.982
Plot#2 (Nic/60/48: Concentration vs MeanValue)	101.214	1.697	0.495	296.879	0.993

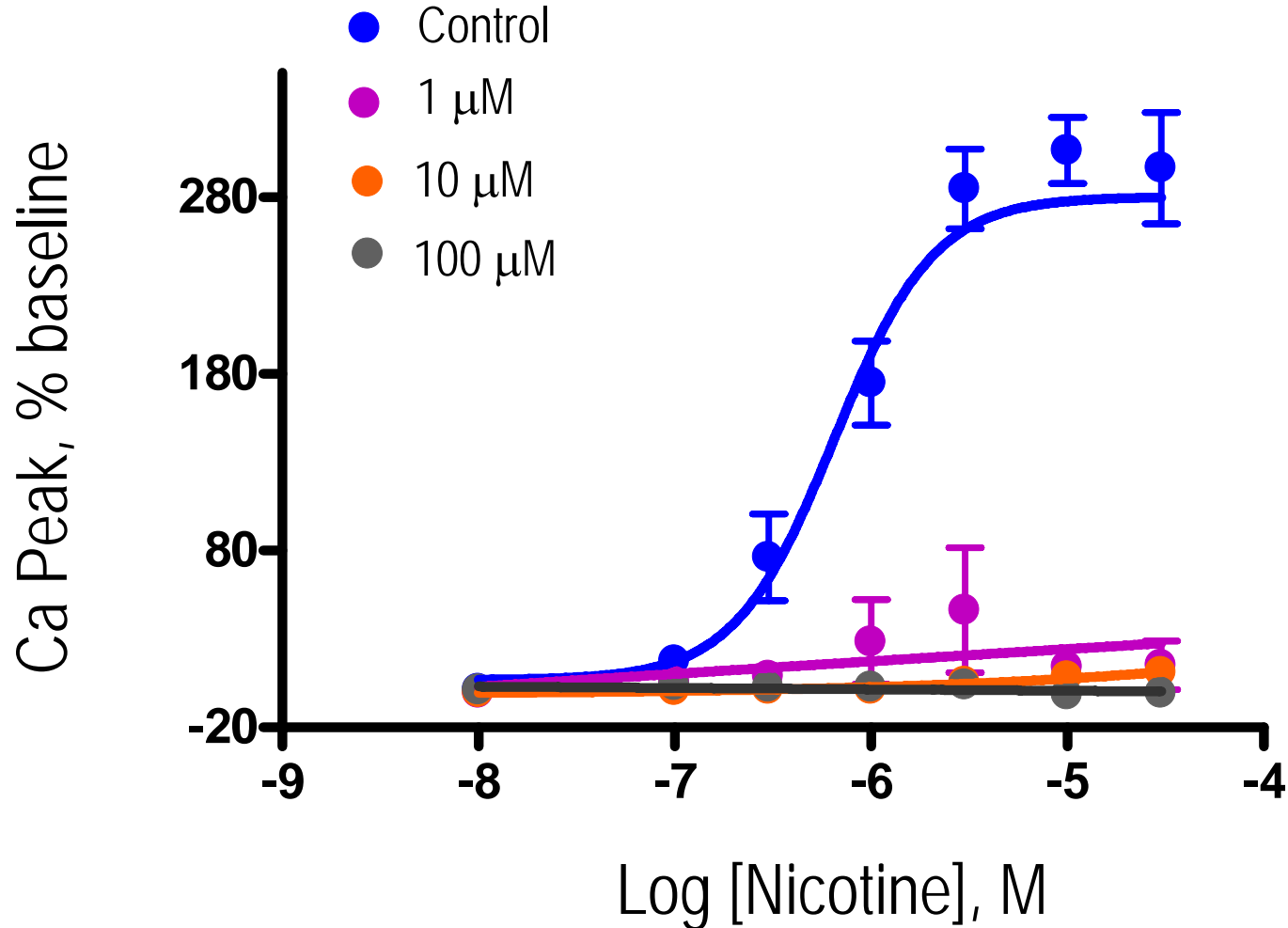
Dihydro- β -erythroidine pretreatment



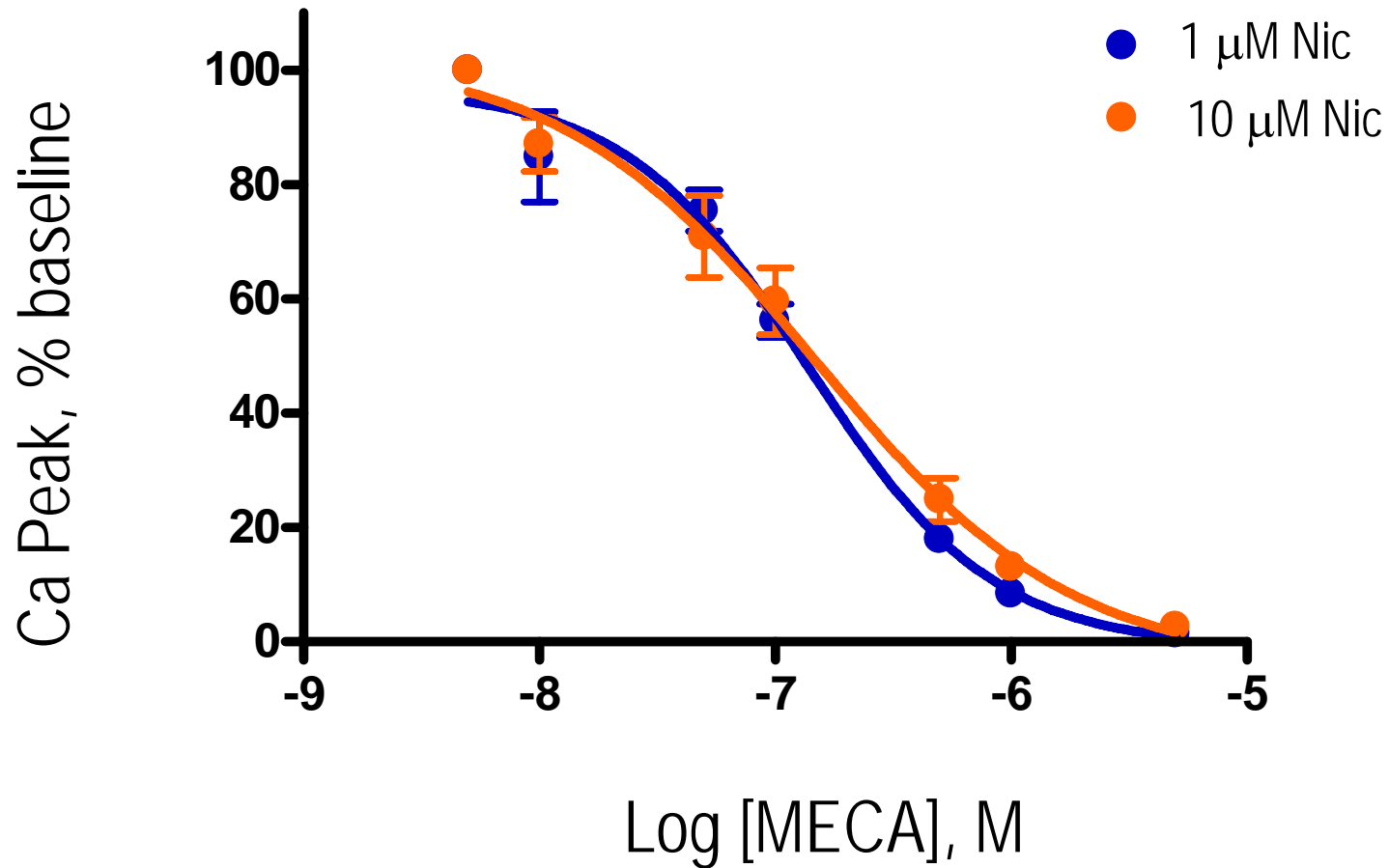
Inhibition Curve



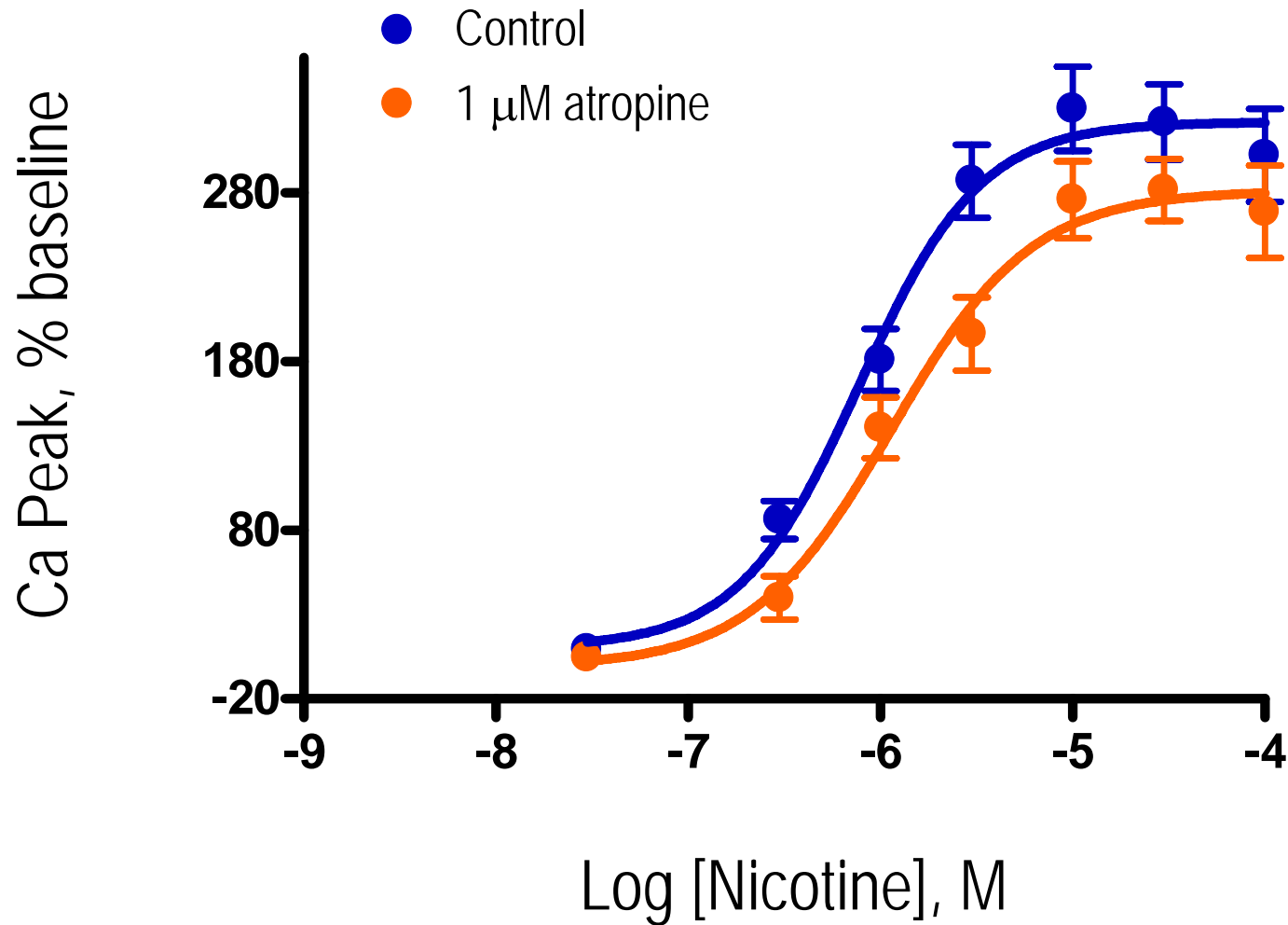
Mecamylamine pretreatment



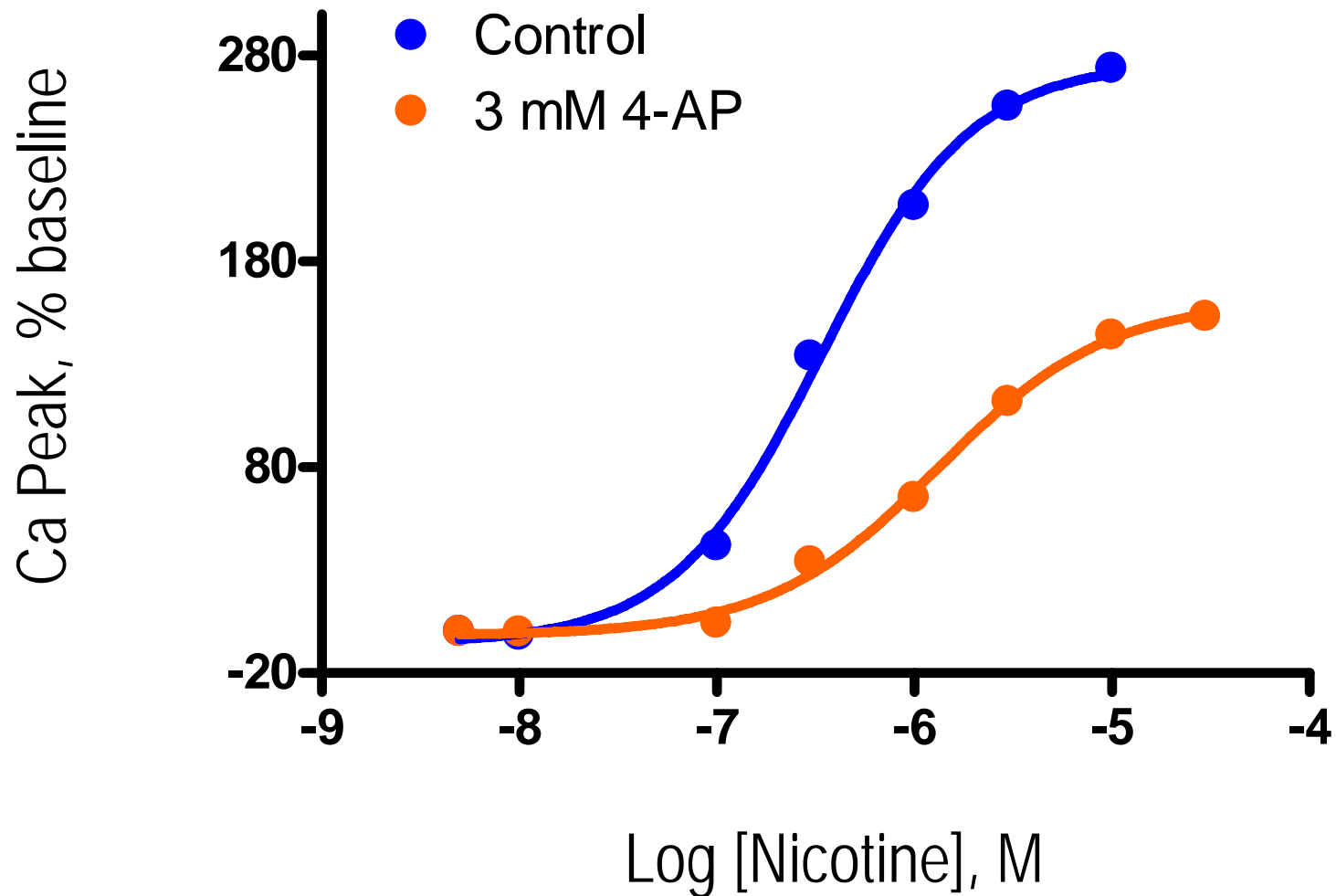
Inhibition Curve



Atropine Effect

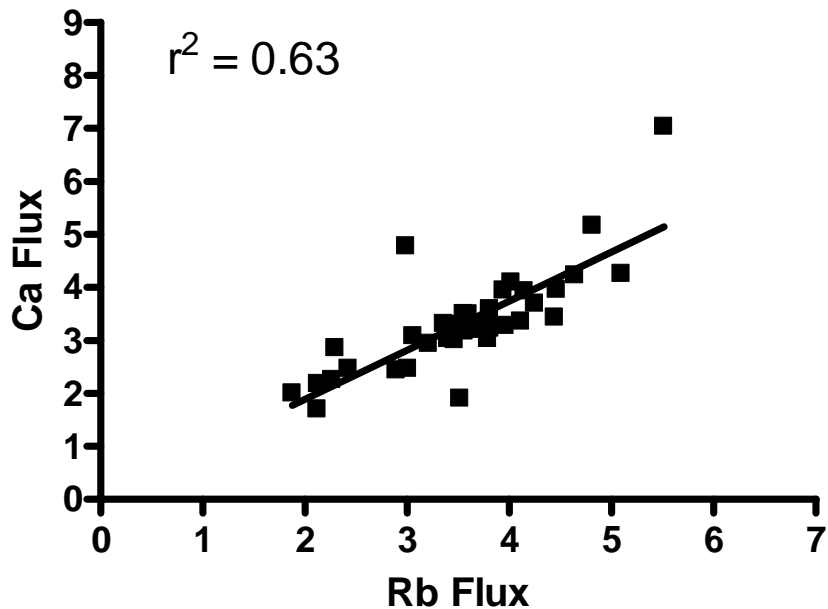


4-Aminopyridine Effect

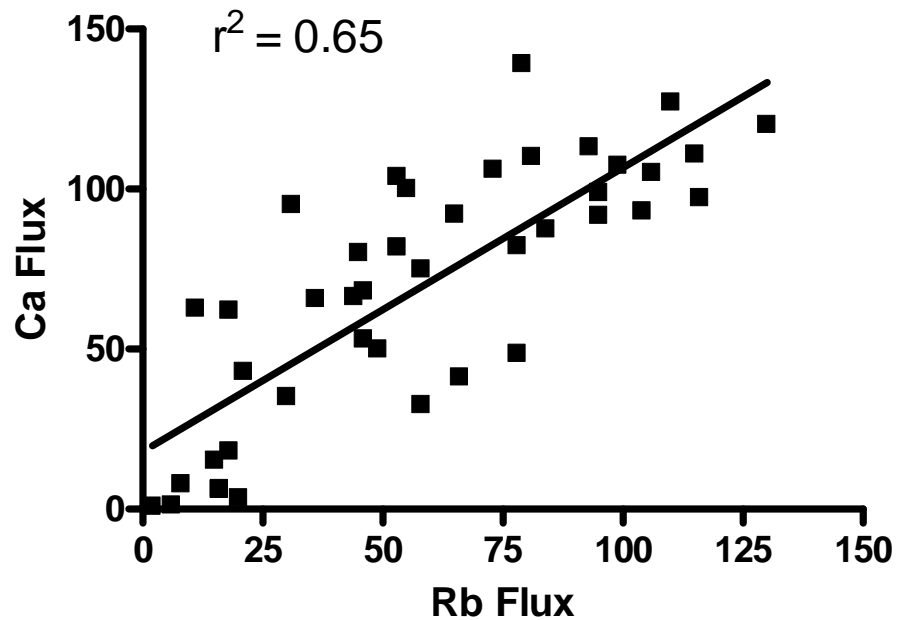


Comparison with Rubidium Flux

EC50



E_{max}



- Lower Na and higher Ca in buffer enhance the Ca response.
- Other factors affect Ca response include: cell seeding density, cell culture time, host cells, and receptor subtypes.
- The Nic-mediated Ca flux was
 - not affected by atropine treatment
 - inhibited by DH β E
 - inhibited by MECA
 - decreased by 4-AP
 - correlated well with Rubidium flux assay
- FLIPR Calcium 4 Assay is a reliable measure of functional activity of α 4 β 2 NNR in SH-EP1 cells.

- Guerra, Leslie-Anne
 - Kiser, Melanie
- Sadieva, Khalima
 - Yocum, Sarah