The signalling and selectivity of α-adrenoceptor agonists for the human α2A, α2B and α2C-adrenoceptors and comparison with human α1 and β-adrenoceptors.

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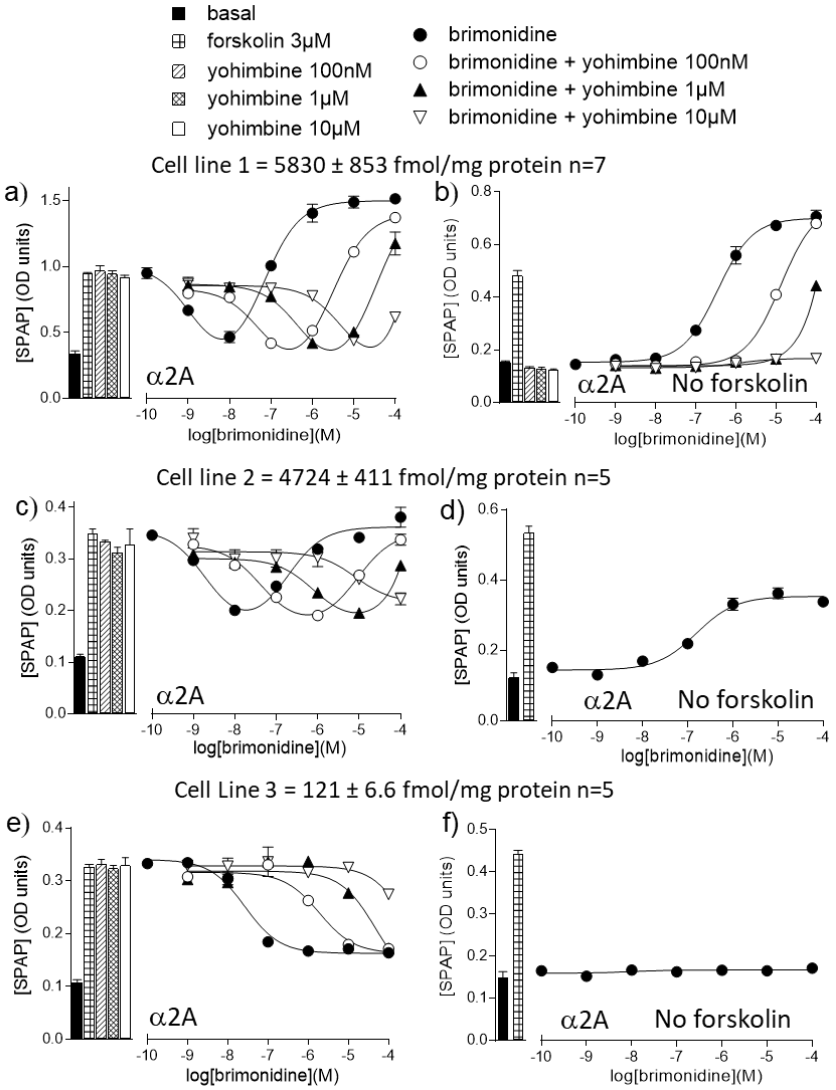
Supplementary Figure 1 - responses in CHO-α2A lines of different expression levels



CHO-α2A	CRE-SPAP (with forskolin)					CRE-SPAP (without forskolin)		
	Log IC_{50} (Gi)	Log EC_{50} (Gs)	% inhibition	n		Log EC_{50} (Gs)	% 3μM forskolin	n
Cell line 1 α2A receptor expression level = 5830 fmol/mg protein								
brimonidine	-8.94 ± 0.05	-7.07 ± 0.04		26		-6.67 ± 0.06	160.8 ± 9.6	11
para-amino-clonidine	-8.74 ± 0.12	-6.81 ± 0.15		8		-6.55 ± 0.10	37.6 ± 4.0	12
clonidine	-8.18 ± 0.04	-6.35 ± 0.12		20			<5%	9
naphazoline	-7.79 ± 0.07		83.1 ± 3.6	16		No response		5
Cell line 2 α2A receptor expression level = 4724 fmol/mg protein								
brimonidine	-8.73 ± 0.10	-6.59 ± 0.10		12		-6.64 ± 0.12	56.1 ± 5.8	9
para-amino-clonidine	-8.51 ± 0.26	-6.29 ± 0.32		4		-6.84 ± 0.11	12.9 ± 4.1	4
clonidine	-8.04 ± 0.08		55.8 ± 2.5	17		No response		4
naphazoline	-7.24 ± 0.07		69.4 ± 5.8	3		ND		
Cell line 3 α2A receptor expression level = 121 fmol/mg protein								
brimonidine	-7.45 ± 0.02		74.5 ± 1.6	12		No response		3
para-amino-clonidine	-7.11 ± 0.18		67.5 ± 4.8	4		No response		4
clonidine	-6.50 ± 0.06		42.9 ± 3.9	12		No response		4

naphazoline	-6.38 ± 0.42		24.8 ± 4.3	3		ND		

ND – not determined

CRE-SPAP production in CHO-α2A cells in response to brimonidine in the absence and presence of yohimbine in 3 cell lines with different levels of α2A-adrenoceptor expression. a), c) and e) are in the presence of 3 μM forskolin and b), d) and f) in the absence of forskolin. Cell line 1 is from the main manuscript. Bars represent basal CRE-SPAP production and that in response to 3 μM forskolin alone. Data points are mean ± sem of triplicate determinations.

Table of data obtained in CHO-α2A cell lines with biphasic log IC_{50} and EC_{50} values from CRE-SPAP production in presence of forskolin, or in the cases of inhibition only, log IC_{50} and % inhibition from the 3 μM forskolin control. Values are mean ± sem of n determinations.

The log K_D values for yohimbine are

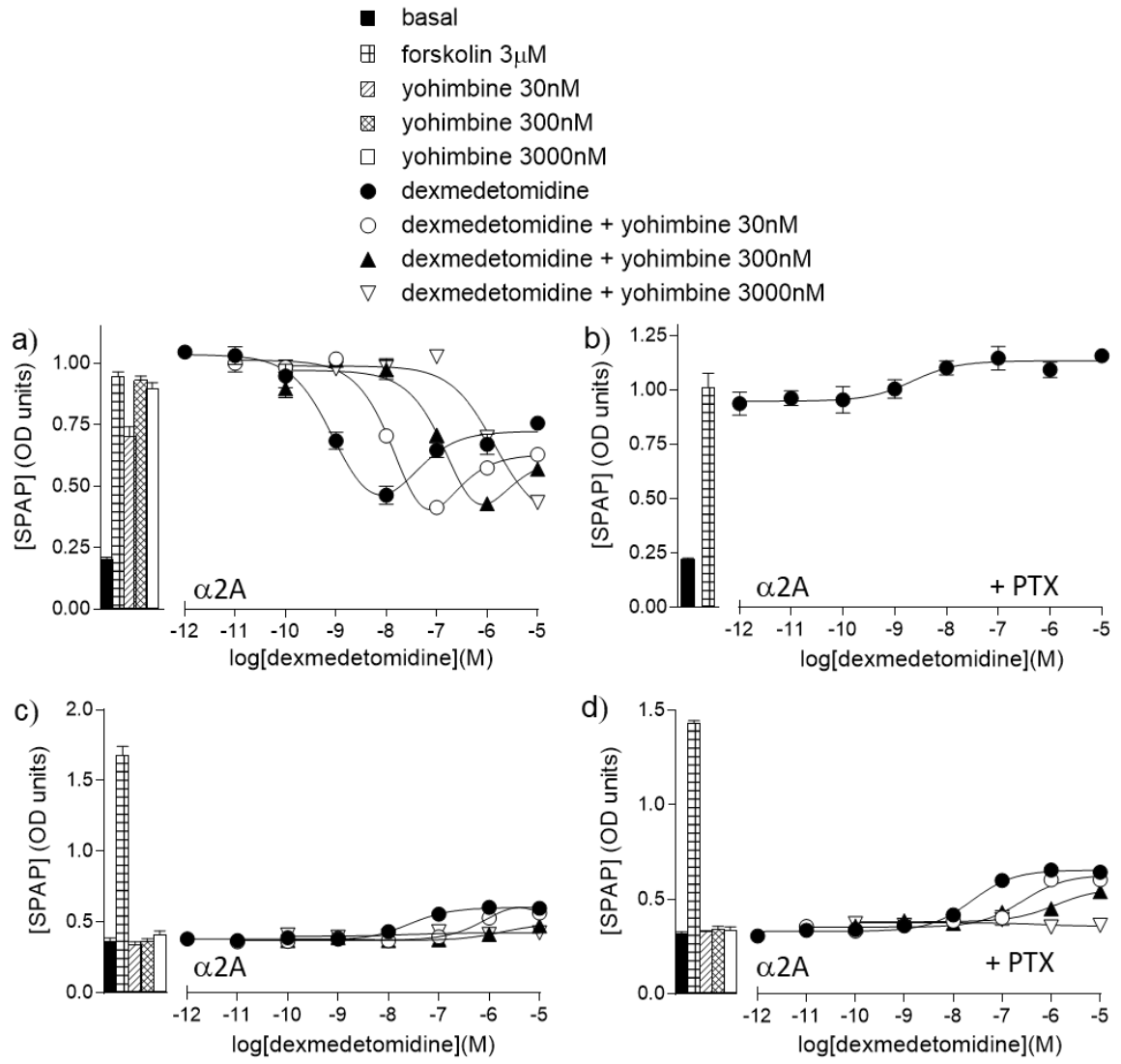
a) -8.45 ± 0.03 n=15 (Gi) and -8.65 ± 0.04 n=13 (Gs); b) -8.61 ± 0.06 n=14 (Gs)
c) -8.22 ± 0.07 n=9 (Gi) and -8.64 ± 0.06 n=9 (Gs);
e) -8.56 ± 0.07 n=10 (Gi)

Receptor expression levels were determined from Bmax from ^{3}H-rauwolscine whole cell binding. For the cell line 1 (main manuscript cell line), the Bmax was determined from ^{3}H-rauwolscine saturation binding (Proudman et al., 2022. Pharmacol Res Perspect. 10(2):e00936. doi: 10.1002/prp2.936). For cell lines 2 and 3, as ^{3}H-rauwolscine (stereoisomer of yohimbine) had been determined to have the same affinity (K_D) as its counterpart yohimbine (Proudman et al., 2022), the Bmax was determined from yohimbine competition curves using the equation:

$$\text{bound ligand} = \frac{\text{Bmax} \times [^3\text{H-rauwolscine}]}{[^3\text{H-rauwolsine}] + K_D \text{ yohimbine}}.$$

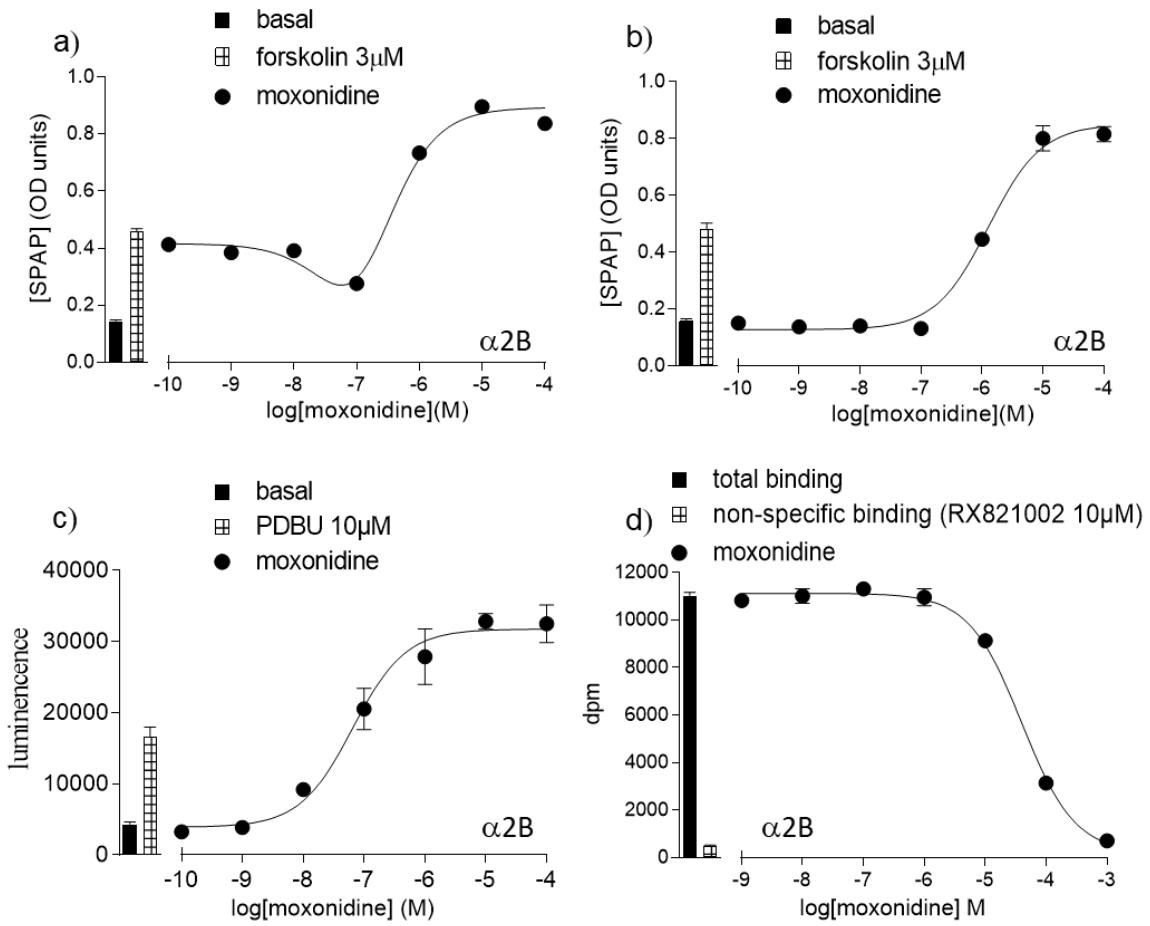
The protein content was determined by the method of Lowry et al., (1951; J. Biol. Chem. 193: 265-275).

Supplementary Figure 2 - dexmedetomidine responses in CHO-α2A cells



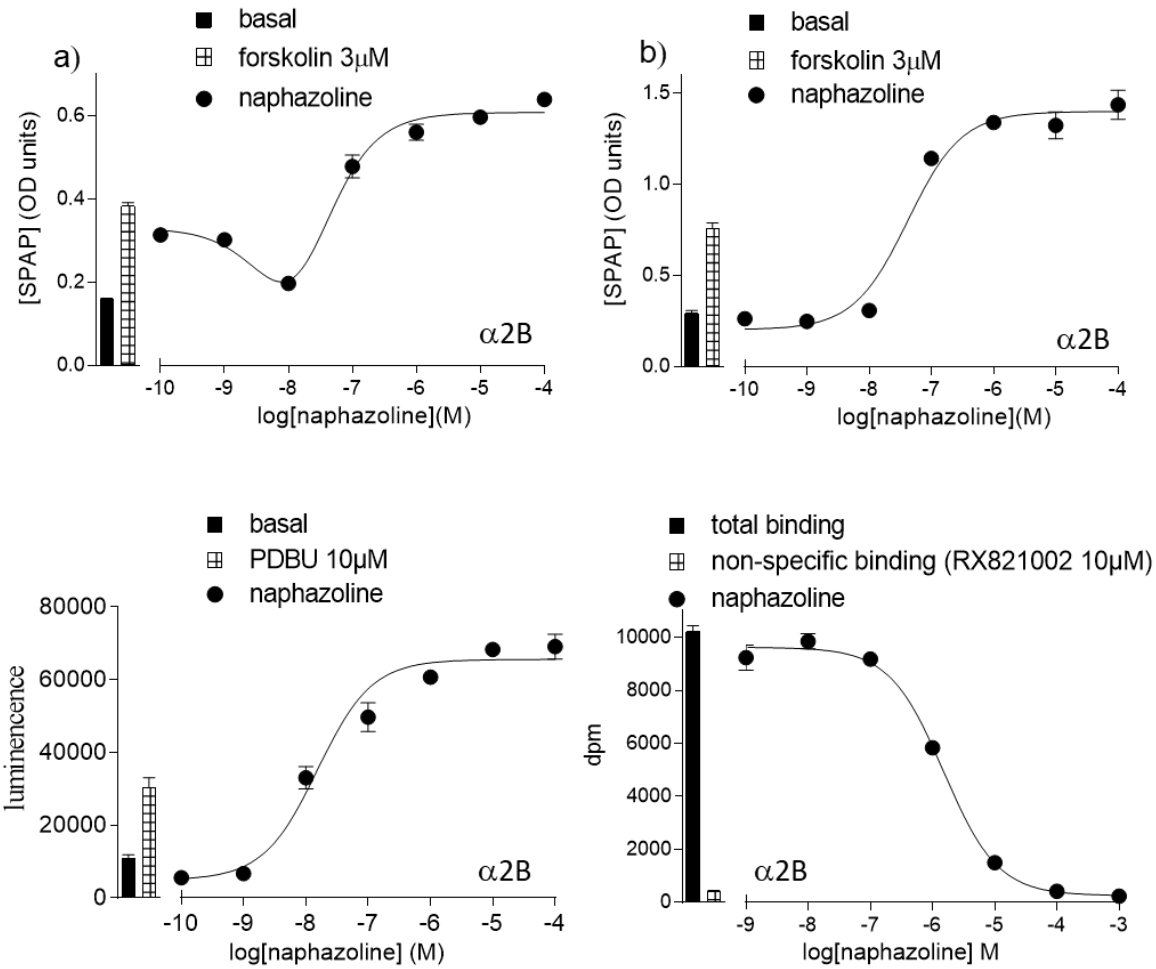
CRE-SPAP in CHO-α2A cells in response to dexmedetomidine in the absence and presence of yohimbine. a) in the presence of 3 μM forskolin, b) in the presence of 3 μM forskolin after 24 hrs PTX pre-treatment, c) in the absence of forskolin and d) in the absence of forskolin after 24 hrs PTX pre-treatment. Bars represent basal CRE-SPAP production, that in response to 3 μM forskolin alone, and that in response to yohimbine 30 nM, 300 nM and 3000 nM alone. Data points are mean ± sem of triplicate determinations.
The log K_D values obtained for yohimbine were a) -8.60 ± 0.04, n=17 (Gi), Schild slope 1.11 ± 0.07 n=5, -8.78 ± 0.06, n=10 (Gs); c) -8.77 ± 0.14, n=9 and d) -8.57 ± 0.10, n=13.

Supplementary Figure 3 - moxonidine responses in CHO-α2B cells



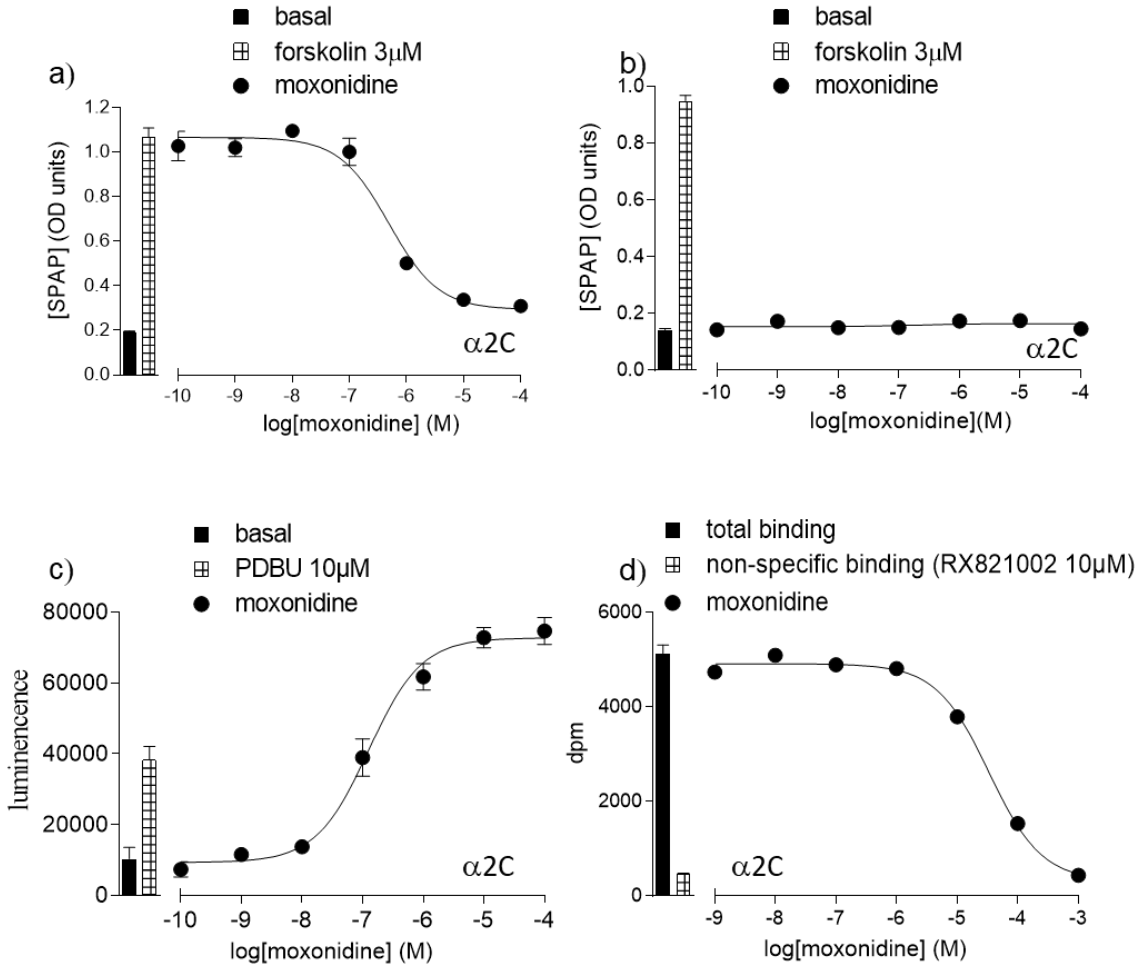
Responses to moxonidine in CHO-α2B cells. a) CRE-SPAP production in the presence of 3 μM forskolin, and b) CRE-SPAP production in the absence of forskolin. Bars represent basal CRE-SPAP production and that in response to 3 μM forskolin alone. c) ERK1/2-phosphorylation. Bars represent basal ERK1/2-phosphorylation and that in response to 10 μM PDBU. and d) inhibition of ^{3}H-rauwolscine binding in whole CHO-α2B cells. Bars represent total binding and non-specific binding as determined by 10 μM RX821002. The concentration of ^{3}H-rauwolscine in this experiment was 0.60 nM.
Data points are mean ± sem of triplicate determinations.

Supplementary Figure 4 - naphazoline responses in CHO-α2B cells



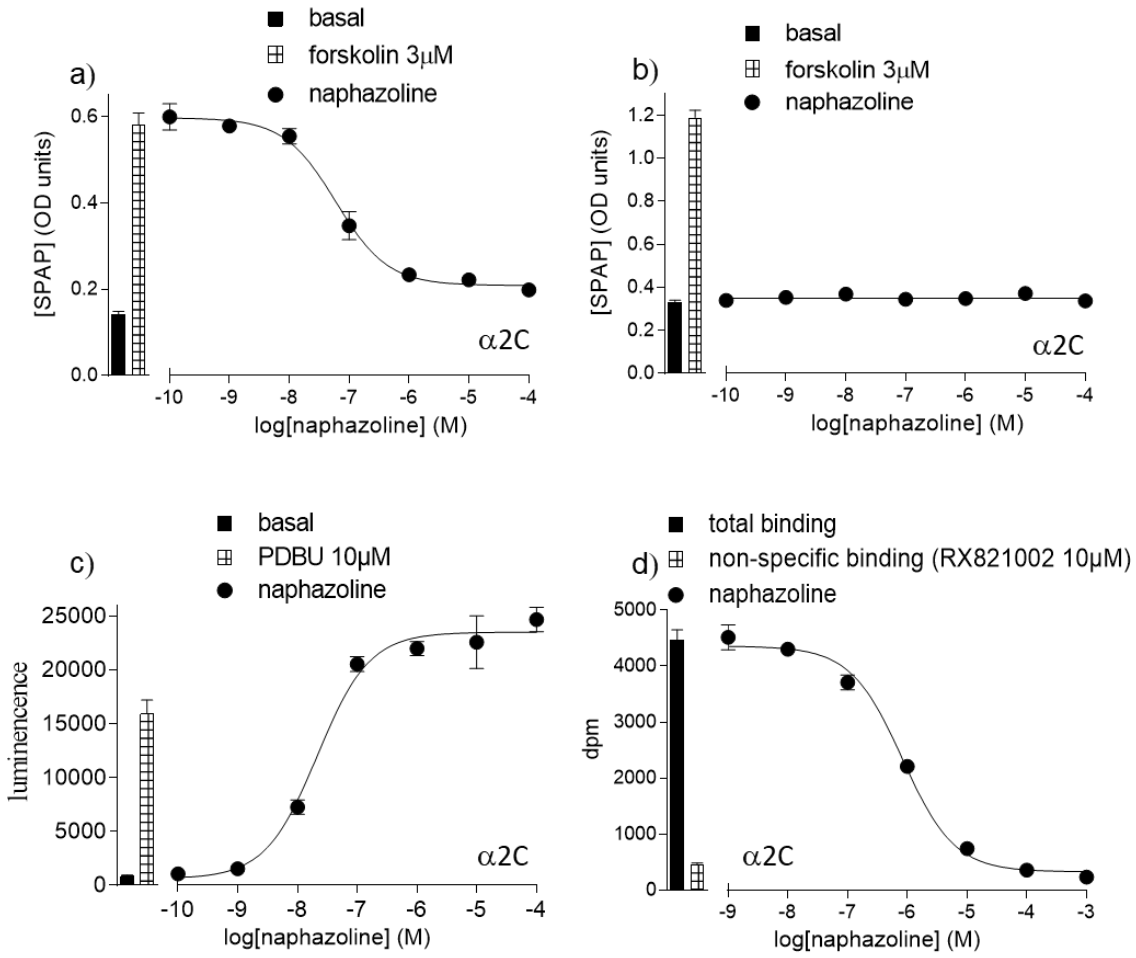
Responses to naphazoline in CHO-α2B cells. a) CRE-SPAP production in the presence of 3 μM forskolin, and b) CRE-SPAP production in the absence of forskolin. Bars represent basal CRE-SPAP production and that in response to 3 μM forskolin alone. c) ERK1/2-phosphorylation. Bars represent basal ERK1/2-phosphorylation and that in response to 10 μM PDBU and d) inhibition of ^{3}H-rauwolscine binding in whole CHO-α2B cells. Bars represent total binding and non-specific binding as determined by 10 μM RX821002. The concentration of ^{3}H-rauwolscine in this experiment was 0.56 nM.
Data points are mean ± sem of triplicate determinations.

Supplementary Figure 5 - moxonidine responses in CHO-α2C cells



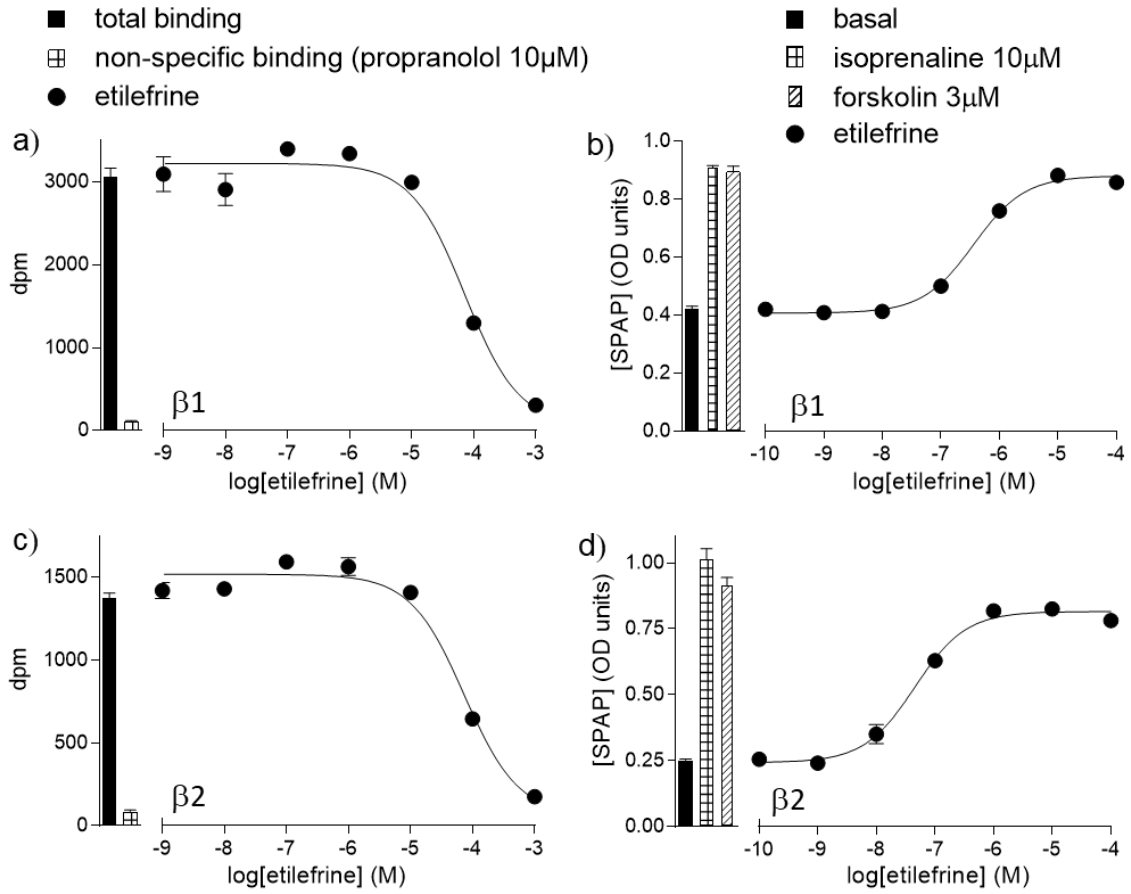
Responses to moxonidine in CHO-α2C cells. a) CRE-SPAP production in the presence of 3 μM forskolin, and b) CRE-SPAP production in the absence of forskolin. Bars represent basal CRE-SPAP production and that in response to 3 μM forskolin alone. c) ERK1/2-phosphorylation. Bars represent basal ERK1/2-phosphorylation and that in response to 10 μM PDBU and d) inhibition of ^{3}H-rauwolscine binding in whole CHO-α2C cells. Bars represent total binding and non-specific binding as determined by 10 μM RX821002. The concentration of ^{3}H-rauwolscine in this experiment was 0.60 nM.
Data points are mean ± sem of triplicate determinations.

Supplementary Figure 6 - naphazoline responses in CHO-α2C cells



Responses to naphazoline in CHO-α2C cells. a) CRE-SPAP production in the presence of 3 μM forskolin, and b) CRE-SPAP production in the absence of forskolin. Bars represent basal CRE-SPAP production and that in response to 3 μM forskolin alone. c) ERK1/2-phosphorylation. Bars represent basal ERK1/2-phosphorylation and that in response to 10 μM PDBU and d) inhibition of ^{3}H-rauwolscine binding in whole CHO-α2C cells. Bars represent total binding and non-specific binding as determined by 10 μM RX821002. The concentration of ^{3}H-rauwolscine in this experiment was 0.60 nM.
Data points are mean ± sem of triplicate determinations.

. Supplementary Figure 7 - etilefrine responses in CHO-β1 and CHO-β2 cells



Responses to etilefrine a) and b) CHO-β1 cells and c) and d) CHO-β2 cells.
a) and c) inhibition of ^{3}H-CGP12177 whole cell binding. Bars represent total binding and non-specific binding as determined by 10 μM propranolol. The concentration of ^{3}H-CGP12177 in these experiments was 0.71 nM.
b and d) CRE-SPAP production in the absence of forskolin. Bars represent basal CRE-SPAP production, that in response to 10 μM isoprenaline or 3 μM forskolin alone.
Data points are mean ± sem of triplicate determinations.

Supplementary table 1 – radioligand binding studies

Ligands (in alphabetical order), with supplier, catalogue number and maximum concentration used in binding assays. The K_D values obtained from ^{3}H-CGP12177 whole cell binding in CHO-β1 and CHO-β2 cells are also given. Values represent mean ± s.e.m. of n separate experiments. Bromocriptine also had high affinity for α1-subtypes as measured by ^{3}H-prazosin whole cell binding: α1A -8.73 ± 0.06 (n=5); α1B -7.96 ± 0.07 (n=5); α1D -7.31 ± 0.15^{ep}, n=9.

	Supplier and catalogue number	Maximum concentration		^{3}H-CGP12177 whole cell binding				
ligand				CHO-β1	n		CHO-β2	n
A61603	Tocris – 1052	100μM		no binding	5		no binding	5
adrenaline	Sigma – E4642	10mM		-4.87 ± 0.06	8		-5.97 ± 0.06	7
allyphenyline	Sigma – SML1484	1mM		No binding	6		No binding	6
amitraz	Sigma – 45323	100μM		no binding	5		no binding	5
atipamezole	Sigma – A9611	30μM		IC_{50}>-4.5	5		IC_{50}>-4.5	5
BHT920	Tocris - 2759	1mM		IC_{50}>-3	5		IC_{50}>-3	5
BHT933	Tocris - 2758	1mM		no binding	5		no binding	5
bromocriptine	Tocris - 0427	10μM		no binding	5		-5.85 ± 0.21^{ep}	5
brimonidine	ARK - AK35795	1mM		no binding	5		no binding	5
buspirone	Sigma – B7148	1mM		IC_{50}>-3	5		IC_{50}>-3	5
chloroethylclonidine	Sigma – B003	100μM		no binding	5		no binding	5
cirazoline	Sigma – C223	1mM		-4.89 ± 0.08	6		-5.26 ± 0.09	6
clonidine	Sigma – C7897	1mM		no binding	5		no binding	5
detomidine	Sigma - 34265	100μM		IC_{50}>-4	6		IC_{50}>-4	6
dexmedetomidine	Sigma – SML0956	100μM		IC_{50}>-4	5		IC_{50}>-4	5
dihydroergotamine	Tocris - 0457	100μM		IC_{50}>-4	6		-5.25 ± 0.01	6
dobutamine	Sigma – D0676	1mM		-5.36 ± 0.05	5		-5.74 ± 0.06	5
dopamine	Sigma – H8502	10mM		-3.57*			-3.93*	
etilefrine	Sigma - 285749	1mM		-4.70 ± 0.04	5		-4.96 ± 0.04	5
ephedrine	ARK – AK390	1mM		-3.82 ± 0.03^{app}	5		-4.55 ± 0.04	5
fenoterol	Sigma – F1016	1mM		-5.04*			-7.03*	
formoterol	Tocris – 1448	100μM		-6.11*			-8.63*	
guanabenz	Sigma – G110	100μM		IC_{50}>-4	5		IC_{50}>-4	5
guanfacine	Sigma – G1043	100μM		IC_{50}>-4	5		IC_{50}>-4	5

isoprenaline	Sigma - I5627	1mM		-6.06*			-6.64*	
medetomidine	Tocris - 5160	100µM		no binding	5		no binding	5
metaraminol	Sigma – M4778	1mM		-4.78 ± 0.04	5		-4.62 ± 0.03	5
methoxamine	Sigma – M6524	1mM		-4.45 ± 0.09	5		-5.22 ± 0.08	5
methyldopa	Tocris – 0584	100µM		No binding	5		No binding	5
α-methylnorepinephrine	Sigma – SML0675	1mM		-5.49 ± 0.05	5		-5.00 ± 0.04	5
midodrine	Sigma – M8277	1mM		No binding	5		No binding	5
moxonidine	Sellakchem – S2066	1mM		No binding	5		No binding	5
naphazoline	Sigma – 70170	1mM		IC_{50}>-3	5		IC_{50}>-3	5
noradrenaline	Sigma – A0937	10mM		-5.43 ± 0.03	9		-4.74 ± 0.07	9
octopamine	Tocris – 2242	1mM		-3.91*			-4.03*	
oxymethazoline	Tocris - 1142	100µM		IC_{50}>-4	5		IC_{50}>-4	5
para-amino-clonidine	Sigma – A0779	100µM		No binding	5		No binding	5
R-phenylephrine	Tocris - 2838	1mM		-4.10 ± 0.09	5		-4.66 ± 0.07	5
rilmenidine	Tocris - 0790	100µM		IC_{50}>-4	5		IC_{50}>-4	5
RWJ52353	Tocris - 3935	100µM		No binding	5		No binding	5
salbutamol	Sigma – S5013	1mM		-4.68*			-6.01*	
salmeterol	Tocris – 1660	100µM		-5.73*			-9.26*	
ST-91	Tocris – 2638	1mM		No binding	5		No binding	5
synephrine	Sigma – S0752	1mM		-3.68 ± 0.02[app]	5		-4.19 ± 0.05	5
T-CG 1000	Tocris – 5021	100µM		IC_{50}>-4	5		IC_{50}>-4	5
tetrahydrozoline	Sigma – T4264	1mM		No binding	5		No binding	5
tizanidine	Sellakchem – S1437	100µM		No binding	5		No binding	5
UK14304	Tocris – 0425	100µM		No binding	5		No binding	5
xylazine	Sigma – X1251	1mM		No binding	5		No binding	5
xylometazoline	Sigma – X6000	1mM		IC_{50}~-3.5	5		IC_{50}>-3	5

[app] = apparent affinity. The maximum concentration of competing ligand inhibited most but not all of specific binding. An IC_{50} was determined by extrapolating the curve assuming that all specific binding would be inhibited if a higher concentration of competing ligand were possible.

[ep] = early plateau, the competing ligand did not fully inhibit specific binding and the inhibition curve reached a plateau of maximal inhibition of binding. The specific binding inhibited by bromocriptine 68.4 ± 1.7% in the CHO-β2 cells and 71.2 ± 3.4% in the CHO-α1D cells

*from Baker (2010). Br. J. Pharmacol. 160: 148-161

Supplementary table 2 – CRE-SPAP functional data

CRE-SPAP responses in CHO-β1 cells, CHO-β2 cells and CHO-CRE-SPAP cells (i.e. parental cell line without any transfected receptor) in alphabetical order of agonist. The maximum concentration used in CRE-SPAP assays is also given. Log EC_{50} values are given (in absence of forskolin) with % of 10 μM isoprenaline maximum response obtained. Ligands were also assessed for inhibitory responses in the presence of 3 μM forskolin – no responses were seen. For ligands that stimulated a response, but the top of the concentration response was not obtained with the highest concentration of agonist, are given as % response at the maximum concentration of agonist used. Values are mean ± sem of n separate experiments.

		CHO-β1 cells					CHO-β2 cells					CRE-SPAP cells			
ligand	maximum	Log EC_{50} % 10μM isop	n	Log IC_{50}	n		Log EC_{50} % 10μM isop	n	Log IC_{50}	n		Log EC_{50} % 10μM isop	n	Log IC_{50}	n
		No forskolin		With forskolin			No forskolin		With forskolin			No forskolin		With forskolin	
A61603	100μM	9.4 ± 5.0%	5	No resp	5		48.4 ± 13.0%	5	No resp	5		No resp	5	No resp	5
adrenaline	100μM	-6.76 ± 0.15 103.7 ± 4.1%	11	No resp	9		-7.43 ± 0.20 101.9 ± 3.5%	10	No resp	8		No resp	7	No resp	7
allyphenyline	100μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
amitraz	10μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
atipamezole	10μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
BHT920	100μM	No resp	5	No resp	5		57.0 ± 5.8%	5	No resp	5		No resp	5	No resp	5
BHT933	100μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
bromocriptine	1μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
brimonidine	100μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
buspirone	100μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
chloroethylclonidine	10μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
cirazoline	100μM	9.5 ± 4.7%	6	No resp	5		15.4 ± 5.2%	7	No resp	7		No resp	7	No resp	7
clonidine	100μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
detomidine	10μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
dexmedetonidine	10μM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
dihydroergotamine	10μM	11.0 ± 5.3%	5	No resp	6		16.2 ± 3.9%	6	No resp	6		No resp	6	No resp	6
dobutamine	100μM	-6.71 ± 0.14 106.4 ± 5.9%	11	No resp	6		-6.56 ± 0.06 100.1 ± 2.4%	9	No resp	6		No resp	6	No resp	6
dopamine	1mM	-5.44 ± 0.03 105.5 ± 6.0%	6	No resp	6		-5.60 ± 0.08 103.1 ± 7.5%	6	No resp	5		No resp	6	No resp	6
etilefrine	100μM	-6.53 ± 0.10 94.7 ± 5.3%	6	No resp	6		-7.22 ± 0.06 93.2 ± 5.2%	6	No resp	6		No resp	6	No resp	6

ephedrine	1mM	-5.21 ± 0.15 50.3 ± 12.7%	3	No resp	3		-6.13 ± 0.07 93.1 ± 3.9%	7	No resp	3		No resp	3	No resp	3
fenoterol	100µM	-7.72 ± 0.05 109.8 ± 8.8%	6	No resp	6		-9.82 ± 0.06 96.6 ± 5.7%	6	No resp	7		No resp	7	No resp	7
formoterol	10µM	-8.83 ± 0.15 101.3 ± 4.0	9	No resp	6		-11.14 ± 0.17 99.3 ± 1.5	10	No resp	7		No resp	6	No resp	6
guanabenz	10µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
guanfacine	100µM	27.4 ± 7.2%	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
isoprenaline	100µM	-7.68 ± 0.19 104.0 ± 0.19%	7	No resp	8		-7.90 ± 0.15 99.9 ± 3.0%	11	No resp	8		No resp	10	No resp	10
medetomidine	10µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
metaraminol	100µM	-6.37 ± 0.10 98.7 ± 7.6%	6	No resp	6		-6.35 ± 0.08 90.0 ± 5.9%	6	No resp	6		No resp	6	No resp	6
methoxamine	100µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
methyldopa	10µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
α-methylnorepin ephrine	100µM	-7.06 ± 0.17 107.5 ± 4.6%	10	No resp	6		-6.86 ± 0.11 107.1 ± 2.3%	6	No resp	6		No resp	6	No resp	6
midodrine	100µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
moxonidine	100µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
naphazoline	100µM	No resp	7	No resp	5		No resp	7	No resp	6		No resp	6	No resp	6
noradrenaline	100µM	-7.13 ± 0.23 109.7 ± 4.8%	11	No resp	9		-6.66 ± 0.13 101.9 ± 3.5	10	No resp	5		No resp	5	No resp	5
octopamine	1mM	-5.70 ± 0.10 101.9 ± 6.5%	7	No resp	6		-5.20 ± 0.04 68.4 ± 5.4%	7	No resp	6		No resp	6	No resp	6
oxymethazoline	100µM	No resp	6	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
para-amino-clonidine	10µM	No resp	6	No resp	6		No resp	6	No resp	6		No resp	6	No resp	6
R-phenylephrine	100µM	-5.84 ± 0.16 89.7 ± 7.8%	8	No resp	6		-7.13 ± 0.07 98.9 ± 3.2%	9	No resp	6		No resp	6	No resp	6
rilmenidine	10µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
RWJ52353	10µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
salbutamol	100µM	-6.45 ± 0.06 103.7 ± 10.2%	5	No resp	5		-8.80 ± 0.13 100.5 ± 5.0%	7	No resp	6		No resp	7	No resp	7
ST-91	100µM	No resp	5	No resp	5		No resp	6	No resp	6		No resp	6	No resp	6
synephrine	100µM	-5.29 ± 0.14 95.0 ± 2.1%	8	No resp	5		-6.21 ± 0.07 97.3 ± 6.1%		No resp	6		No resp	6	No resp	6
T-CG 1000	10µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
tetrahydrozoline	100µM	No resp	5	No resp	5		No resp	6	No resp	6		No resp	6	No resp	6
tizanidine	100µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5

UK14304	10µM	No resp	5	No resp	5		9.2 ± 3.8%	5	No resp	5		No resp	5	No resp	5
xylazine	100µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5
xylometazoline	100µM	No resp	5	No resp	5		No resp	5	No resp	5		No resp	5	No resp	5

No resp = no response