Establishing relative efficacies of analgesic μ opioid receptor agonists in adenylyl cyclase activity and β -arrestin2 recruitment assays

Clara Dieterle[1], Samuel Singleton[1], Daniel T. Baptista-Hon[1,2], Tim G. Hales[1]

[1] Institute of Academic Anaesthesia, Division of Systems Medicine, School of Medicine, Ninewells Hospital, University of Dundee, Dundee, UK
[2] Faculty of Medicine, Macau University of Science and Technology, Macau SAR, China

Introduction and Aim

Opioids are the most effective analgesics for treating moderate and severe acute pain. However, side effects occur with the use of opioids including respiratory depression and tolerance (1). Opioids recruit upon activation of μ opioid receptors (μORs) β-arrestin2, which has been implicated in the detrimental side effects (2).

Some opioids have been reported to be biased in favour of G protein-signalling and hence suggested to cause fewer side effects (3). However, assessment of biased opioids might have been confounded by the presence of spare receptors, making partial agonists appear full agonists in G protein-signalling.

Relative efficacies and potencies of DAMGO, fentanyl, oxycodone, 5-aminosotonitazene, bromadoline and bromadol have been assessed to examine their abilities as recruiters of β-arrestin2 and inhibitors of cAMP accumulation under conditions of full and partial availability of μORs as well as compromised β-arrestin2 recruitment in Chinese hamster ovary cells.

Methods

cAMP
- Quantification of cAMP levels
  - On forskolin-stimulated cells, luciferase-based

βarr2
- Quantification of β-arrestin2 recruitment
  - Enzyme fragment complementation assay

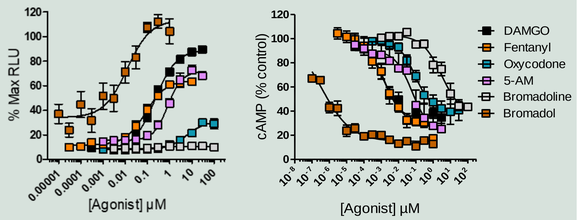
βFNA
- Reducing available receptors using β-FNA
  - Irreversible μOR antagonist

cmpd 101
- Limiting β-arrestin2 recruitment with cmpd101
  - GRK2/3 inhibitor

Results

cAMP, βarr2a

β-arrestin2 recruitment and inhibition of cAMP accumulation in CHO cells overexpressing μORs



	β-arrestin2				cAMP			
Agonist	% max RLU	EC_{50} (nM)	Hill slope	*n*	cAMP (% forskolin max)	IC_{50} (nM)	Hill slope	*n*
DAMGO	84 ± 2	343 ± 54	1 ± 0.1	21	65 ± 5	3 ± 2	1.1 ± 0.1	11
Fentanyl	53 ± 4 *	116 ± 30 *	1.6 ± 0.3	12	77 ± 6	2 ± 1	0.8 ± 0.1	7
Oxycodone	27 ± 6 *	10242 ± 3501 *	1 ± 0.2	6	58 ± 8	103 ± 27 *	1.1 ± 0.2	5
5-AM	56 ± 5 *	937 ± 232	1.5 ± 0.2	6	71 ± 9	62 ± 21 *	1 ± 0.2	5
Bromadoline	4 ± 0.4 *	8478 ± 7017 *	1.8 ± 1.4	6	68 ± 3	6667 ± 3226 *	1 ± 0.2	5
Bromadol	103 ± 13 *	52 ± 37 *	0.8 ± 0.2	5	62 ± 25	7 ± 7 *	1.2 ± 0.6	5

cAMP, βarr2, βFNA

β-arrestin2 recruitment and inhibition of cAMP accumulation in CHO cells with increasingly restricted receptor reserve

	β-arrestin2									
	0 nM			1 nM			10 nM			
Agonist	% max RLU	EC_{50} (nM)	Hill slope	% max RLU	EC_{50} (nM)	Hill slope	% max RLU	EC_{50} (nM)	Hill slope	
Fentanyl	100 ± 7	116 ± 30	1.6 ± 0.3	50 ± 8 *	266 ± 105	1 ± 0.3	21 ± 3 *	607 ± 546	1.9 ± 0.5	
Oxycodone	100 ± 27	10242 ± 3501	1.1 ± 0.2	39 ± 11	7887 ± 2263	0.9 ± 0.2	20 ± 6 *	63214 ± 59355	1.4 ± 0.3	
5-AM	100 ± 9	937 ± 232	1.7 ± 0.1	76 ± 22	2114 ± 414 #	1.3 ± 0.1	39 ± 15 *	4202 ± 1364 #	0.9 ± 0.2	

- Efficacy reduced following restricted μOR availability

	cAMP							
	0 nM				100 nM			
Agonist	cAMP (% forskolin max)	IC_{50} (nM)	Hill slope	*n*	cAMP (% forskolin max)	IC_{50} (nM)	Hill slope	*n*
DAMGO	65 ± 5	3 ± 2	1.1 ± 0.1	11	71 ± 10	105 ± 66 #	1.2 ± 0.4	5
Fentanyl	77 ± 6	2 ± 1	0.8 ± 0.1	7	80 ± 9	2304 ± 2262 #	1.4 ± 0.6	5
Oxycodone	58 ± 8	103 ± 27	1.1 ± 0.2	5	47 ± 12	2343 ± 1170 #	1.9 ± 0.7	5
5-AM	71 ± 9	62 ± 21	1 ± 0.2	5	58 ± 13	180 ± 89	1.8 ± 0.3	5
Bromadoline	68 ± 3	6667 ± 3226	1 ± 0.2	5	12 ± 5 *	n.d.	n.d.	6
Bromadol	62 ± 25	7 ± 7	1.2 ± 0.6	5	65 ± 15	5 ± 5	1.7 ± 0.6	5

- Only potency reduced following μOR depletion

cAMP, βarr2, βFNA

Comparison of correlations between μOR overexpression and depletion

- Depleting receptor reserve reveals correlation between the efficacies of μOR agonists

cmpd 101, βarr2

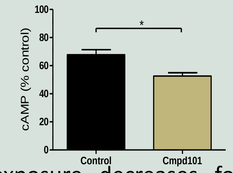
GRK2/3 inhibition limits β-arrestin2 recruitment

	β-arrestin2							
	0 μM				30 μM			
Agonist	% max RLU	EC_{50} (nM)	Hill slope	*n*	% max RLU	EC_{50} (nM)	Hill slope	*n*
DAMGO	75 ± 3	239 ± 40	1.1 ± 0.1	15	14 ± 2 *	563 ± 68 #	1.7 ± 0.3	5
Bromadol	103 ± 13	52 ± 37	0.8 ± 0.2	5	10 ± 2 *	17 ± 4	1.6 ± 0.5	5

- Cmpd101 abolishes β-arrestin2 recruitment of DAMGO and bromadol

cAMP, cmpd 101

Inhibition of cAMP accumulation in CHO cells following exposure to compound 101



- Cmpd101 exposure decreases forskolin-stimulated cAMP levels

	cAMP							
	0 μM				30 μM			
Agonist	cAMP (% forskolin max)	IC_{50} (nM)	Hill slope	*n*	cAMP (% forskolin max)	IC_{50} (nM)	Hill slope	*n*
DAMGO	65 ± 5	3 ± 2	1.1 ± 0.1	11	94 ± 15 *	6 ± 3	1.3 ± 0.1	5
Fentanyl	77 ± 6	2 ± 1	0.8 ± 0.1	7	88 ± 16	40 ± 38	0.9 ± 0.2	5
Oxycodone	58 ± 8	103 ± 27	1.1 ± 0.2	5	99 ± 11 *	275 ± 87	1.1 ± 0.2	5
5-AM	71 ± 9	62 ± 21	1 ± 0.2	5	111 ± 13 *	128 ± 87	0.8 ± 0.1	5

- Cmpd101 exposure increases efficacies of agonists

Conclusion

- Biased agonism may be compromised by differences in available receptor number
- Reducing μOR availability and limiting β-arrestin2 recruitment is proving crucial for a better characterization of μOR agonists and more accurate estimate of agonist efficacy

References

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