MOLPHARM/2020/119453

Supplemental data

A truncated six transmembrane splice variant MOR-1G enhances expression of the full-length seven transmembrane mu opioid receptor through heterodimerization

Tiffany Zhang, Jin Xu, and Ying-Xian Pan

Supplemental Figure 1. Opioid receptor binding in CHO cells transfected with the 6TM variants.

Equal amount of the indicated 6TM variant constructs in pcDNA3, as well as mMOR-1 (a positive control) and pcDNA3 alone (a negative control), was transfected into CHO cells using Lipofectamine reagent (Invitrogen). Cell membranes were prepared 48 hrs after transfection. Equal amount of membrane proteins (100 µg) from each transfection was used for opioid receptor binding with [^{3}H]Diprenorphine (1.8 nM), [^{3}H]DAMGO (2 nM) and [^{125}I]IBNtxA (0.15 nM), as described in Materials and Methods. Specific binding was defined as the difference between total binding and nonspecific binding at the presence of 10 µM levallorphan.

Supplemental Figure 2. Opioid receptor binding in Tet-Off CHO cells stably transfected with mMOR-1G.

The Tet-Off CHO cells stably transfected with mMOR-1G was established by transfecting mMOR-1G construct in pTRE2Hyg vector and selecting by 0.6 mg/ml of hygromycin. The expression of mMOR-1G in the stable transfectants with or without doxycycline was confirmed by RT-qPCR (data not shown). Cell membranes treated with

100 ng/ml of doxycycline or without doxycycline were prepared and used for opioid receptor binding with [^{3}H]Diprenorphine (2.1 nM) and [^{125}I]IBNtxA (0.13 nM), as described in Materials and Methods. A membrane from CHO cells stably expressing mMOR-1 was used as a positive control. Specific binding was defined as the difference between total binding and nonspecific binding at the presence of 10 μM levallorphan.

Supplemental Figure 3. [^{35}S]GTPγS binding in Tet-Off CHO cells stably transfected with mMOR-1G.

Equal amount of cell membrane proteins (60 μg) from mMOR-1G Tet-Off CHO cells treated with 100 ng/ml of doxycycline or without doxycycline was used in [^{35}S]GTPγS binding assays with DAMGO (10 μM), morphine (10 μM) and IBNtxA (10 μM), as described in Materials and Methods. A membrane from CHO cells stably expressing mMOR-1 was used as a positive control.

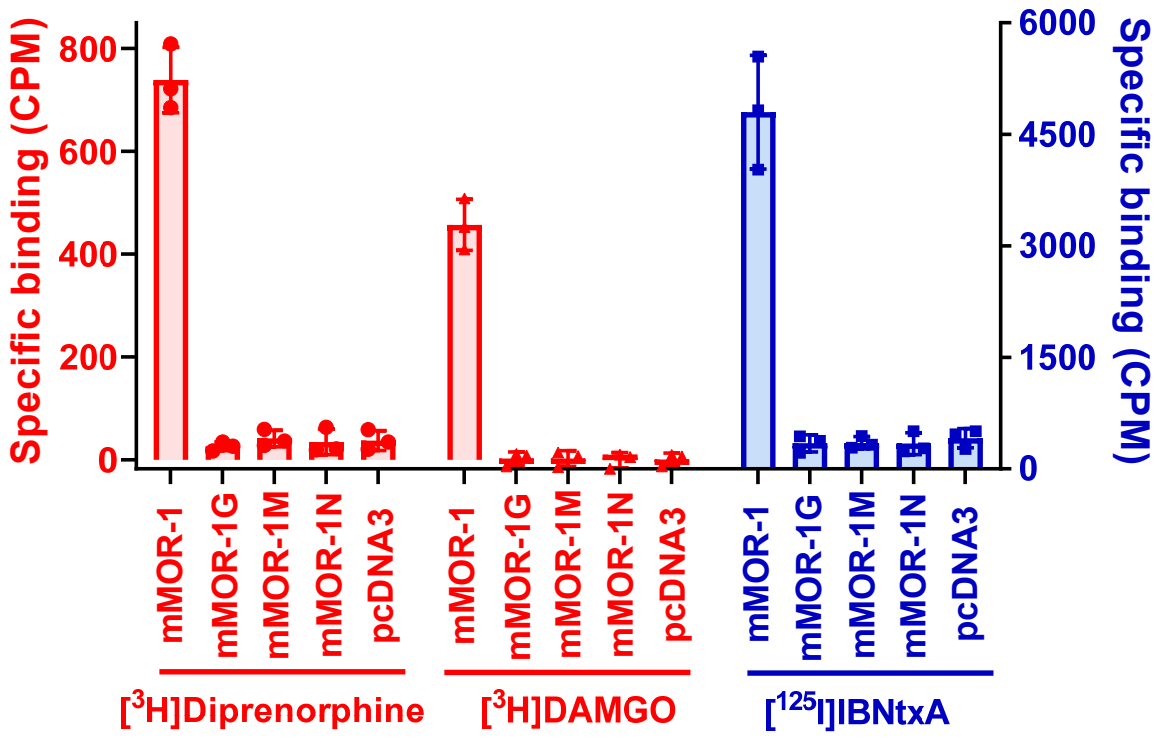
Specific binding (CPM)
800
600
400
200
0
6000
4500
3000
1500
0
Specific binding (CPM)
mMOR-1
mMOR-1G
mMOR-1M
mMOR-1N
pcDNA3
mMOR-1
mMOR-1G
mMOR-1M
mMOR-1N
pcDNA3
mMOR-1
mMOR-1G
mMOR-1M
mMOR-1N
pcDNA3
[3H]Diprenorphine
[3H]DAMGO
[125I]IBNtxA

Fig. S1.

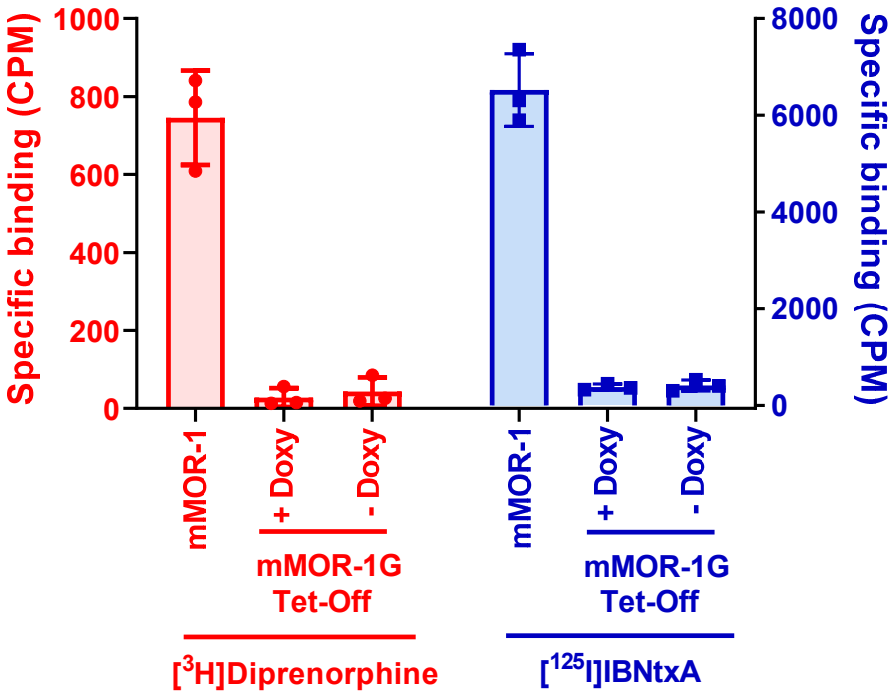
Specific binding (CPM)
1000
800
600
400
200
0
8000
6000
4000
2000
0
Specific binding (CPM)
mMOR-1
+ Doxy
- Doxy
mMOR-1
+ Doxy
- Doxy
mMOR-1G
Tet-Off
mMOR-1G
Tet-Off
[3H]Diprenorphine
[125I]IBNtxA

Fig. S2.

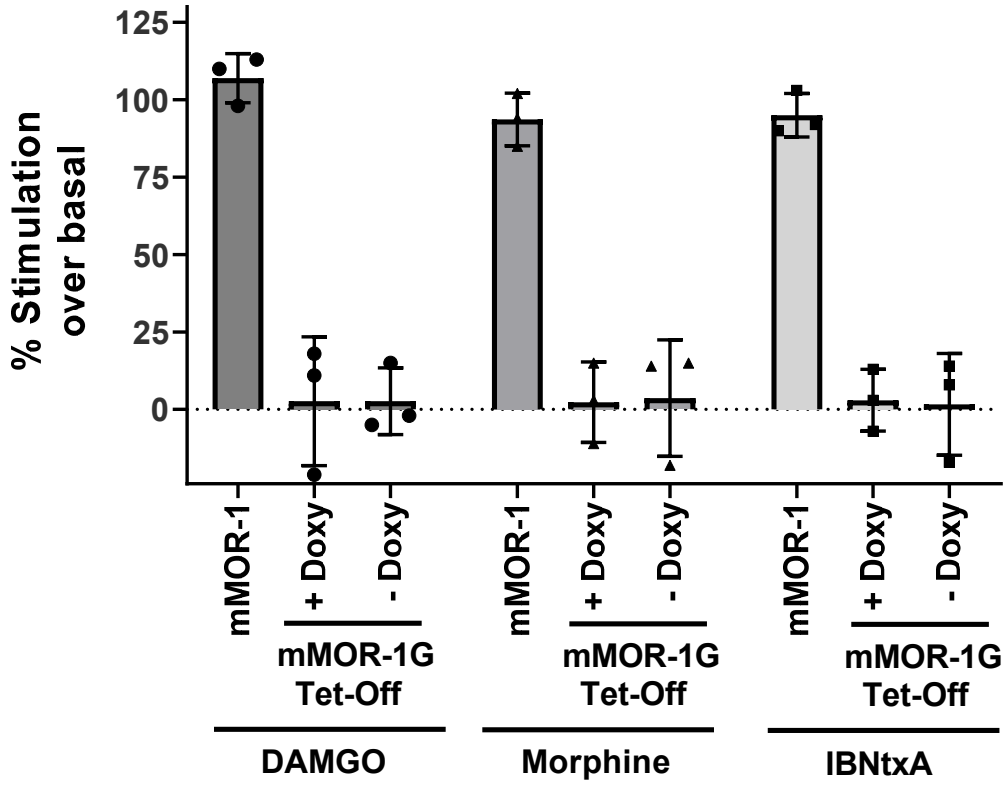
% Stimulation over basal
125
100
75
50
25
0
mMOR-1
+ Doxy
- Doxy
mMOR-1G Tet-Off
DAMGO
Morphine
IBNtxA

Fig. S3.