FGFR-Tyr766 regulates the A2AR-FGFR interaction.

(A) Evaluation of the importance of the two tyrosine residues (Y730 and Y766) present in the FGFR minimal domain of interaction. The different mutants were tested by GST pull-down (PD) using a GST-A2AR recombinant protein as indicated in Figure 1.

(B) Signals corresponding to the pull-down results from (A) were quantified and statistical differences were determined by Student's t test. *: p<0.05 vs wild type.
The synergistic activation of MAPK pathway occurs at the MEK1/2 level and is specific. MEK1/2 and ERK1/2, but not c-Raf, are phosphorylated by the synergistic action of CGS21680 and aFGF. Cells were incubated with aFGF and/or CGS21680 and samples were analyzed by immunoblotting using phospho-specific antibodies directed against c-Raf (pSer259), MEK1/2 (pSer217/Ser221) and ERK1/2.
Absence of effect of the A2AR-FGFR interaction on the activity of the two receptors. (A) aFGF does not significantly affect A2AR-induced cAMP production. Cells were incubated with CGS21680 (1 µM) in the absence or presence of aFGF (100 ng/ml) for 10 min, lysed, and cAMP content of cell lysates analyzed by ELISA. Results were analyzed statistically as described elsewhere. ***: p<0.001 vs. no addition.

(B) CGS21680 does not significantly influence FGF-induced FGFR phosphorylation. Cells were incubated with aFGF (100 ng/ml) in the absence or presence of CGS21680 (1 µM) and samples were analyzed by immunoblotting using a phospho-tyrosine specific antibody. The signals corresponding to phosphorylated FGFR were quantified, normalized to total FGFR, and analyzed statistically as described elsewhere. *: p<0.05 vs. no addition.
Co-application of CGS21680 (20 nM) and aFGF (10 ng/ml) enhances NMDA currents in D2 MSNs. (A) Plot of EPSC amplitude as a function of time before and after the bath application of CGS21680 and aFGF in a D2 MSN recorded in whole cell mode in the presence of NBQX (20 µM) to block AMPA receptors and bicuculline (10 µM) to block GABAa receptors. The somatic membrane potential was held at -50 mV to diminish Mg2+ block of the NMDA receptors. Insets are representative current traces before and after CGS21680 and aFGF application; note the slow kinetics of the currents (stimulus artifact was removed digitally). Below the amplitude plot is a plot of series resistance during the recording. (B) Plot of averaged results from a sample of 6 D2 MSNs (mean, S.E.M.)