Arrestin-Mediated Activation of p38 MAPK: Molecular Mechanisms and Behavioral Consequences

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Abstract Studies of kappa opioid receptor signaling mechanisms during the last decade have demonstrated that agonist activation of the receptor results in Gβγ-dependent signaling and distinct arrestin-dependent signaling events. Gβγ-dependent signaling results in ion channel regulation causing neuronal inhibition, inhibition of transmitter release, and subsequent analgesic responses. In contrast, arrestin-dependent signaling events result in p38 MAPK activation and subsequent dysphoric and proaddictive behavioral responses. Resolution of these two branches of signaling cascades has enabled strategies designed to identify pathway-selective drugs that may have unique therapeutic utilities.

Keywords Kappa opioid receptor • Dynorphin • Arrestin • p38 MAPK

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Abbreviations

ASK1	Apoptosis signal-regulating kinase 1
β2AR	β_2-Adrenergic receptor
CRF	Corticotropin-releasing factor
JNK	c-Jun N-terminal Kinase
ERK1/2	Extracellular signal-regulated kinase
GRK	G-protein receptor kinase
GPCRs	G-protein-coupled receptors
GIRK, Kir3	G-protein-gated inwardly rectifying potassium channel
GFAP	Glial fibrillary acidic protein
KOR	Kappa opioid receptor
rKOR	Rodent KOR
hKOR	Human KOR
MAPK	Mitogen-activated protein kinase
MAP3K5	Mitogen-activated protein kinase kinase kinase 5
PKC	Protein kinase C
5HT	Serotonin, 5-hydroxytryptamine

1 Introduction

The concept that arrestin association with G-protein-coupled receptors (GPCRs) does more than cause homologous receptor desensitization grew out of the realization that arrestin recruitment by the β_2-adrenergic receptor (β2AR) resulted in Src tyrosine kinase activation and phosphorylation of extracellular signal-regulated kinase (ERK1/2) (Daaka et al. 1997; Luttrell et al. 1999). Subsequently, Miller et al. (2003) found that the cytokine receptor US28 (a GPCR encoded by the human cytomegalovirus) activates p38 mitogen-activated protein kinase (MAPK) through a G-protein receptor kinase (GRK) and arrestin-dependent mechanism. In addition, Sun et al. (2002) found that the chemotaxic response of HeLa and HEK cells to cytokines mediated by the CXCR4 receptor also required p38 MAPK activation through receptor phosphorylation and arrestin-3 recruitment. Parallel studies showed that the GPCR-arrestin signaling complex activates c-Jun N-terminal kinase (JNK), also through a physical scaffolding mechanism (McDonald et al. 2000; Breitman et al. 2012). These observations lead to the concept that the arrestins can form a scaffold that physically links the GPCR to the three different MAPK signaling cascades: ERK1/2, p38 MAPK, and JNK (Burack and Shaw 2000; Pearson et al. 2001; DeWire et al. 2007).

The steps linking arrestin activation to p38 MAPK phosphorylation have not been fully visualized, but a requirement for apoptosis signal-regulating kinase 1 (ASK1), also known as mitogen-activated protein kinase kinase kinase 5 (MAP3K5), was suggested by the ability of dominant-negative mutant of ASK1 to block p38 MAPK activation (Sun et al. 2002). A plausible model for p38

activation suggests that activated arrestin forms a scaffold containing the required sequential cascade of the three kinases typically involved in a MAPK activation: a MAPKKK (possibly ASK1) activating a MAPKK (possibly MEK3 or MEK6), which in turn activates p38 MAPK (Burack and Shaw 2000; Pearson et al. 2001; Dewire et al. 2007). Presumably, arrestin association with the GRK-phosphorylated GPCR induces a structural rearrangement within arrestin–kinase complex, thereby facilitating the sequential phosphorylation reactions. However, the details of this cascade and differences between the cascades in different cell types and subcellular compartments have not yet been resolved.

Arrestin-dependent p38 MAPK activation results in a range of cellular and behavioral responses. In addition to mediating ***chemotaxic*** responses to cytokines (Sun et al. 2002), activation of p38 MAPK via arrestin association regulates ***apoptosis*** in mouse embryonic fibroblasts (Yang et al. 2012) and mediates endothelin-induced cell migration of mouse aortic smooth muscle cells (Morris et al. 2012). Arrestin-mediated p38 activation also induces ***hypertrophy*** and ***proliferation*** of GFAP-immunoreactive astrocytes in the spinal cord and brain (Bruchas et al. 2006; Xu et al. 2007). In addition, we recently found that arrestin-dependent p38 activation plays a key role in the ***behavioral stress response***, and it is the cellular details of this signaling cascade that we would like to summarize in this chapter.

In essence, our studies in mice have shown that:

1. Corticotropin-releasing factor (CRF) is released in the brain and hypothalamus in response to stress exposure.
2. CRF acts broadly in brain to coordinate the physiological, adaptive response to stress necessary for survival.
3. One of the cellular responses to CRF is the stimulated release of the endogenous dynorphin opioid peptides (Land et al. 2008; Bruchas et al., 2009).
4. Dynorphins selectively activate the kappa opioid receptors (KOR), which are Gi/o-coupled GPCRs (Chavkin et al. 1982; Bruchas et al. 2007a, b; Land et al. 2008; Bruchas et al. 2011; Lemos et al. 2012).
5. Sustained KOR activation results in GRK3-mediated phosphorylation of Ser369 in rodent KOR and subsequent arrestin-3 recruitment (Bruchas et al. 2006).
6. The KOR–arrestin complex initiates the phosphorylation and activation of p38α MAPK at multiple sites within the brain (Bruchas et al 2011; Lemos et al. 2012; Schindler et al. 2012).
7. p38α activation at one of these sites (the nerve terminals of the serotonergic neurons projecting from the dorsal raphe nucleus to the ventral striatum) causes the translocation of the serotonin transporter (SERT; SLC6A4) from an endosomal compartment to the nerve terminal surface (Bruchas et al. 2011; Schindler et al. 2012).
8. Increase in surface expression of SERT pumps serotonin (5HT) back into the nerve terminal more efficiently and thereby produces a transient hyposerotonergic state in the ventral striatum (Bruchas et al. 2011; Schindler et al. 2012).

9. The reduction in 5HT tone in the ventral striatum contributes to the stress-induced dysphoria evident as behavioral aversion in the mice exposed to the stressful experience (Land et al. 2008; Bruchas et al. 2011; Schindler et al. 2012).

Evidence supporting this proposed cascade is summarized below.

2 Kappa Opioid Receptors

Kappa opioid receptors are members of the Gi/o-coupled superfamily of G-protein-coupled receptors (Bruchas and Chavkin 2010). Consistent with the data concerning other members of this class, kappa receptor activation results in a broad range of signaling events including membrane-delimited Gβγ-mediated regulation of calcium and potassium conductances. Gβγ released by kappa receptor activation increases G-protein-gated inwardly rectifying potassium channel (Kir3) activation and positively shifts the activation threshold of voltage-sensitive calcium channels (VSCC), thereby inhibiting calcium conductance (Werz and Macdonald 1984; Cherubini and North 1985; Herlitze et al. 1996). The net effect of these membrane-delimited effects on ion conductance is to reduce somatic excitability and calcium influx at the nerve terminal; kappa receptor activation has been shown to presynaptically inhibit the release of a broad range of neurotransmitters through these ionic mechanisms (Grudt and Williams 1995; Simmons and Chavkin 1996).

G-protein stimulation by kappa receptors also activates a variety of kinases including ERK1/2, JNKs, PKC, and p38 MAPKs in receptor-transfected cells, primary cultures of neurons and astrocytes, and in kappa opioid receptor expressing neurons in the brain (see Bruchas and Chavkin 2010). These activated kinases phosphorylate a variety of substrates, including transcription factors to regulate gene expression and various cytoplasmic proteins to affect neuronal physiology (to be described further below).

Again, like other G-protein-coupled receptors, agonist-activated kappa receptors are substrates for GRKs, which phosphorylate specific serine residues in the carboxy-terminal domain of the receptor [i.e., serine-369 in the rodent kappa receptor (rKOR) and the homologous residue serine-358 in the human kappa receptor (hKOR) sequence] (Appleyard et al. 1999; Li et al. 2002; McLaughlin et al. 2003; Schattauer et al. 2012). Arrestin binding to the GRK-phosphorylated kappa receptor sterically inhibits further G-protein activation and results in homologous desensitization of membrane-delimited signaling, but arrestin association is required for the late phase of ERK1/2 activation (Bruchas et al. 2008; McLennan et al. 2008) and for p38 MAPK activation by kappa receptors (Bruchas et al. 2006; Xu et al. 2007). Thus, arrestin binding to kappa receptors shifts agonist signaling from membrane-delimited pathways to alternative effector pathways.

With this wide range of possible cellular signaling responses, it should not be surprising that the ability of a kappa agonist to activate one pathway (its efficacy)

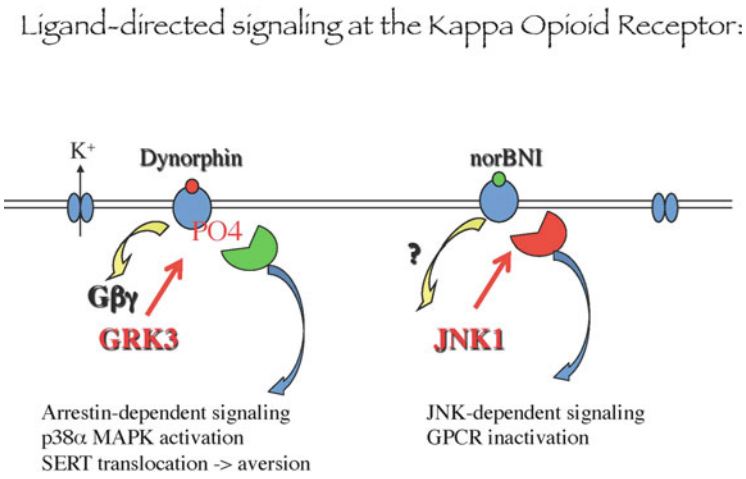

Fig. 1 Ligand-directed signaling differences between kappa opioids. Strong agonists, like dynorphin, activate kappa receptors to stimulate Gβγ-dependent responses including presynaptic inhibition of transmitter release (calcium channel inhibition) and somatic membrane hyperpolarization (potassium channel activation). Sustained kappa receptor activation results in the phosphorylation of specific serine residues in the carboxy-terminal domain of the receptor and subsequent arrestin (green symbol) recruitment. The resulting arrestin activation enables p38 MAPK activation (phosphorylation), and the cellular consequences include astrocyte activation, Kir3 potassium channel phosphorylation (and deactivation), and SERT translocation. At the behavioral level, presynaptic inhibition of transmitter release underlies the analgesic responses, and SERT translocation mediates dysphoria and proaddictive responses. In contrast, long-acting kappa antagonists, like norBNI, cause receptor inactivation through a c-Jun N-terminal kinase (JNK)-dependent mechanism without stimulating Gβγ-dependent responses

does not need to be the same as for all the different pathways. Ligand-directed signaling differences have been documented in other GPCR systems (Urban et al. 2007) (see Chap. 3). Based on these insights, kappa ligands can be conceptually divided into (1) ***strong agonists*** (able to activate all of the Gβγ- and arrestin-dependent signaling events), (2) ***weak agonists*** (able to activate Gβγ-, but not arrestin-dependent signaling events), (3) ***neutral antagonists*** (that bind receptor but do not evoke any signaling responses), and (4) ***collateral agonists*** (that bind to kappa receptors to activate one of the alternative signaling pathways without activating Gβγ-dependent responses) (Fig. 1). ***Arrestin-biased agonists*** (see Chap. 3) at the kappa receptor that activate arrestin-dependent signaling without efficiently activating Gβγ signaling could be postulated by analogy to the parathyroid hormone and angiotensin II receptors (Gesty-Palmer et al. 2009; Violin et al. 2010); however, examples of this type of ligand have not yet been characterized.

Dynorphin peptides, salvinorin A, U50,488, U69,593, and enadoline are prominent members of the first category. Buprenorphine, naloxone, and naltrexone are examples of neutral antagonists (although they lack kappa receptor selectivity). A ligand that activated Gβγ signaling but did not efficiently stimulate GRK would be

expected to activate the membrane-delimited signaling but not the arrestin-dependent responses (Chavkin 2011). 6′GNTI has been suggested as an example of a G-protein-biased kappa receptor agonist that does not recruit arrestin (Rives et al. 2012). An example of this type of pathway-selective ligand in a different receptor system is morphine, which is a strong opioid analgesic acting through mu opioid receptors, but does not efficiently activate arrestin-dependent responses (Dang and Christie 2012). norBNI and JDTic are examples of the latter category; the selective kappa ligands norBNI and JDTic do not activate Gβγ- or arrestin-dependent pathways, but do effectively activate JNK pathways upon kappa receptor binding (Bruchas et al. 2007a, b; Melief et al. 2010).

3 Stress-Induced Release of Dynorphin Increases Phospho-p38 MAPK in a GRK3- and Arrestin-3-Dependent Manner

Efforts to understand opioid receptor tolerance mechanisms entered a new molecular biology phase after the delta opioid receptors were cloned by Kieffer and Evans in 1994, and the mu and kappa sequences were deduced shortly afterwards (Akil et al 1996). In a series of site-directed mutagenesis studies using *Xenopus* oocyte expression, the serine-369 residue in the carboxy-terminal domain was found to be the critical GRK phosphorylation site required for homologous rKOR desensitization (Appleyard et al. 1999). To determine if this phosphorylation event also regulated kappa opioid signaling in vivo, we generated a phospho-selective antibody, KOR-p, that could distinguish phosphorylated KOR-pSer369 from the unphosphorylated receptor (McLaughlin et al. 2003). Importantly, the increase in KOR-p immunoreactivity induced by the kappa agonist U50,488 was not evident in GRK3−/− mice. The selective role of GRK3 (without compensation by other GRK isoforms) was a surprise. Mice lacking GRK3 showed reduced analgesic tolerance to U50,488 (McLaughlin et al. 2003).

The high degree of cellular resolution of the immunohistochemical KOR-p staining provided a new opportunity to detect sites of dynorphin action in the brain, and we next adopted a partial sciatic nerve ligation method previously shown by Porecca and colleagues to evoke endogenous dynorphin release (Wang et al. 2001). We found that KOR-p immunoreactivity was increased in the spinal cord following partial sciatic nerve ligation in wild type, but not in KOR−/−, prodynorphin−/−, or GRK3−/− mice (Xu et al. 2004). Sustained dynorphin release following nerve ligation produced tolerance to the analgesic effects of U50,488, but nerve ligation did not produce tolerance in GRK3−/− or prodynorphin −/− mice (Xu et al. 2004). These results established that kappa opioid receptor desensitization occurred both in vivo and in vitro through a GRK-/arrestin-dependent mechanism.

4 Astrocyte Activation by Dynorphin Occurs Through an Arrestin/p38 MAPK Mechanism

One of the striking features of nerve ligation is that it causes the robust activation of astrocytes, as documented by the increase in number of GFAP-immunoreactive cells in the spinal cord. However, we were surprised to observe that the increased GFAP immunoreactivity was not evident in prodynorphin−/− or GRK3−/− mice and that the activation of astrocytes by nerve ligation could be blocked by the p38 MAPK inhibitor SB 203580 (Xu et al. 2007). Using KOR-transfected AtT20 cells, we found that kappa receptor stimulation increased phospho-p38 immunoreactivity and that the increase could be blocked by a dominant-negative form of arrestin but not evident if kappa receptor phosphorylation was blocked by alanine substitution for Ser369 in KOR (Bruchas et al. 2006). A GRK3-/arrestin-dependent mechanism of p38 activation in astrocytes stimulated by kappa agonists in vivo and in vitro was also documented by confocal imaging and Western blot analysis (Bruchas et al. 2006; Xu et al. 2007). p38 MAPK activation was not evident in ether striatal astrocytes or neurons isolated from KOR−/− or GRK3−/− mice, and cultured striatal astrocytes pretreated with siRNA for arrestin-3 were also unable to activate p38 in response to U50,488 treatment (Bruchas et al. 2006). McLennan et al. (2008) also found that proliferation of immortalized astrocytes in culture could be stimulated by kappa opioids in a Gβγ- and arrestin-dependent manner. They attributed these effects to pERK activation—not p38; however, in a subsequent study, they reported that both ERK and p38 pathways stimulated oligodendrogenesis in a similar culture system (Hahn et al. 2010). Extending these findings, we found that forced swim stress also activates GFAP-immunoreactive astrocytes in hippocampus and cortex by stimulating this dynorphin-KOR-GRK3-arrestin $\Rightarrow$ phospho-p38 MAPK cascade (Messinger and Chavkin unpublished observations).

5 Kappa Receptor Activation of Arrestin/p38 MAPK Regulates the Potassium Channel Kir3

Prior studies showed that tyrosine phosphorylation in the N-terminal cytoplasmic domain of the G-protein-gated inwardly rectifying potassium channel, Kir3.1, facilitates channel deactivation by increasing the intrinsic GTPase activity of the channel (Ippolito et al. 2002, 2005). Dynorphin released during forced swim stress or following sciatic nerve ligation also resulted in tyrosine phosphorylation of Kir3.1 at these regulatory residues (Clayton et al. 2009). Channel phosphorylation in the dorsal horn of the spinal cord of nerve-ligated mice required GRK3 phosphorylation of the kappa opioid receptor, arrestin recruitment, and subsequent p38 MAPK activation (Clayton et al. 2009). Whole cell voltage clamp of AtT20 cells expressing kappa receptors demonstrated that p38 activation reduced the potassium current through a Src kinase-dependent mechanism; the enhanced channel

deactivation could be blocked by the Src inhibitor PP2. Similar mechanisms also regulate Kir3 current in serotonergic neurons of the dorsal raphe nucleus in the brain (Lemos et al. 2012). Acute activation of kappa receptors in these neurons increases potassium conductance through G-protein-gated inwardly rectifying channel, but sustained kappa receptor activation by repeated stress exposure causes channel phosphorylation and subsequent channel inactivation through the arrestin-dependent p38 MAPK mechanism (Lemos et al. 2012).

6 Kappa Receptor Activation of Arrestin/p38 MAPK Activates the Serotonin Transporter

Selective kappa agonists produce feelings of dysphoria in humans and aversion responses in experimental animals (Pfeiffer et al. 1986; Shippenberg and Herz 1986). Stress-induced release of the endogenous dynorphin opioid peptides selectively activates kappa opioid receptors and produces dysphoria in experimental animals (McLaughlin et al. 2006; Bruchas et al. 2007a, b; Land et al. 2008). The dysphoria caused by stress-induced activation of the dynorphin-kappa opioid systems results in a potentiation of the rewarding valence of cocaine and reinstatement of extinguished cocaine drug seeking, which may help explain how stress increases the risk of drug addiction.

We used a conditional gene deletion approach to define the molecular events responsible for these behavioral responses. Using mice having lox-p excision sequences flanking the p38α MAPK, we found that selective inactivation of p38 signaling in serotonergic neurons of the dorsal raphe nucleus blocked defeat-induced social aversion and stress-induced reinstatement of cocaine place preference (Bruchas et al. 2011). In addition, selective excision of p38α MAPK in serotonergic neurons blocked stress-induced potentiation of cocaine place preference (Schindler et al. 2012). These behavioral responses were each caused by stress-induced dynorphin release, kappa opioid receptor activation, GRK3-dependent kappa receptor phosphorylation, and subsequent arrestin recruitment and activation. Previous reports had demonstrated a role for p38 MAPK in the modulation of the plasma membrane serotonin transporter (SERT, SLC6A4) function in vitro (Zhu et al. 2004, 2005; Samuvel et al. 2005), and using a cell-surface biotinylation and Michaelis-Menten kinetic analysis of 5HT transport, we found that stress-induced activation of p38α in serotonergic neurons causes SERT translocation from a cytoplasmic endosomal compartment to the cell surface (Bruchas et al. 2011; Schindler et al. 2012). Although the dorsal raphe sends afferent projections broadly throughout the forebrain, dynorphin-dependent SERT translocation was evident only in the serotonergic projection to the ventral striatum (Schindler et al. 2012). These findings suggest that stress-induced dysphoria mediated by arrestin/p38 MAPK activation is caused by a transient hyposerotonergic state in the nucleus accumbens (Fig. 2).

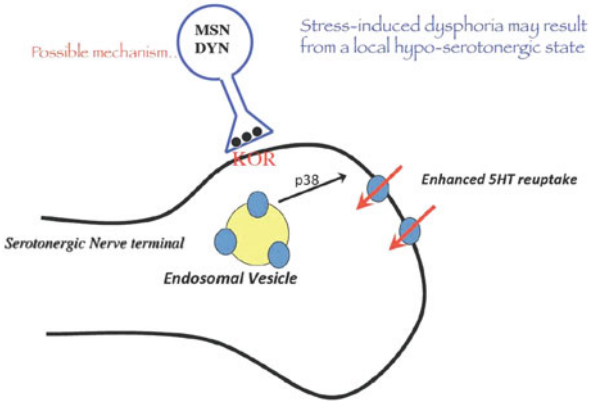

Fig. 2 Stress exposure regulates serotonergic neurotransmission. Dynorphin opioid peptide release from medium spiny neurons (MSN) in the ventral striatum activates kappa opioid receptors (KOR) expressed on the terminals of the serotonergic neurons innervating the nucleus accumbens. Through a G-protein receptor kinase 3 (GRK3)-dependent mechanism, arrestin activates p38α MAPK in the nerve terminals, thereby increasing cell-surface expression of the serotonin transporter. The transient hyposerotonergic state in the nucleus accumbens likely contributes to the dysphoria underlying stress-induced aversion and stress-induced potentiation of the rewarding valence of abused drugs

7 Conclusions

These studies show that kappa receptor activation by either endogenous dynorphin release in vivo or pharmacological activation in vitro causes p38 MAPK activation through a GRK3- and arrestin-dependent mechanism. p38 MAPK is likely to have a broad range of substrates and to regulate a diverse group of processes. Several of these have been identified, but others are plausible. Several important questions have not been resolved, including the characterization of specific signaling steps linking kappa receptor activation of arrestin to p38 MAPK activation and differences in signaling in different cell types and subcellular compartments. Since arrestin/p38 signaling is essential for the dysphoric and proaddictive effects of kappa opioids, but not for their analgesic effects, we expect that pathway-selective kappa agonists, which need to be identified, will have therapeutic advantages.

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References

Akil H, Meng F, Mansour A, Thompson R, Xie GX, Watson S (1996) Cloning and characterization of multiple opioid receptors. NIDA Res Monogr 161:127–40

Appleyard SM, Celver J, Pineda V, Kovoor A, Wayman GA, Chavkin C (1999) Agonist-dependent desensitization of the kappa opioid receptor by G protein receptor kinase and β-arrestin. J Biol Chem 274:23802–23807

Breitman M, Kook S, Gimenez LE, Lizama BN, Palazzo MC, Gurevich EV, Gurevich VV (2012) Silent scaffolds: inhibition of c-Jun N-terminal kinase 3 activity in cell by dominant-negative arrestin-3 mutant. J Biol Chem 287:19653–19664

Bruchas MR, Chavkin C (2010) Kinase cascades and ligand-directed signaling at the kappa opioid receptor. Psychopharmacology (Berl) 210:137–147

Bruchas MR, Macey TA, Lowe JD, Chavkin C (2006) Kappa opioid receptor activation of p38 MAPK is GRK3- and arrestin-dependent in neurons and astrocytes. J Biol Chem 281:18081–18089

Bruchas MR, Land BB, Aita M, Xu M, Barot SK, Li S, Chavkin C (2007a) Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. J Neurosci 27:11614–11623

Bruchas MR, Yang T, Schreiber S, Defino M, Kwan SC, Li S, Chavkin C (2007b) Long-acting kappa opioid antagonists disrupt receptor signaling and produce noncompetitive effects by activating c-Jun N-terminal kinase. J Biol Chem 282:29803–29811

Bruchas MR, Xu M, Chavkin C (2008) Repeated swim-stress induces kappa opioid-mediated activation of ERK1/2 MAPK. Neuroreport 19(14):1417–1422

Bruchas MR, Land BB, Lemos JC, Chavkin C (2009) CRF1-R activation of the dynorphin/kappa opioid system in the mouse basolateral amygdala mediates anxiety-like behavior. PLoS One 4 (12):e8528

Bruchas MR, Schindler AG, Shankar H, Messinger DI, Miyatake M, Land BB, Lemos JC, Hagen C, Neumaier JN, Quintana A, Palmiter RD, Chavkin C (2011) Selective p38alpha MAPK deletion in serotonergic neurons produces stress-resilience in models of depression and addiction. Neuron 71:498–511

Burack WR, Shaw AS (2000) Signal transduction. Hanging on a scaffold. Curr Opin Cell Biol 12:211–216

Chavkin C, James IF, Goldstein A (1982) Dynorphin is a specific endogenous ligand of the kappa opioid receptor. Science 215:413–415

Chavkin C (2011) The therapeutic potential of kappa opioids for treatment of pain and addiction. Neuropsychopharmacology 36:369–370

Cherubini E, North RA (1985) Mu and kappa opioids inhibit transmitter release by different mechanisms. Proc Natl Acad Sci USA 82:1860–1863

Clayton CC, Xu M, Chavkin C (2009) Tyrosine phosphorylation of Kir3 following kappa opioid receptor activation of p38-MAPK causes heterologous desensitization. J Biol Chem 284:31872–31881

Daaka Y, Luttrell LM, Lefkowitz RJ (1997) Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature 390:88–91

Dang VC, Christie MJ (2012) Mechanisms of rapid opioid receptor desensitization, resensitization and tolerance in brain neurons. Br J Pharmacol 165:1704–1716

DeWire SM, Ahn S, Lefkowitz RJ, Shenoy SK (2007) Beta-arrestins and cell signaling. Annu Rev Physiol 69:483–510

Gesty-Palmer D, Flannery P, Yuan L, Corsino L, Spurney R, Lefkowitz RJ, Luttrell LM (2009) A beta- arrestin-biased agonist of the parathyroid hormone receptor (PTH1R) promotes bone formation independent of G protein activation. Sci Transl Med 1:1ra1

Grudt TJ, Williams JT (1995) Opioid receptors and the regulation of ion conductances. Rev Neurosci 6:279–286

Hahn JW, Jagwani S, Kim E, Rendell VR, He J, Ezerskiy LA, Wesselschmidt R, Coscia CJ, Belcheva MM (2010) Mu and kappa opioids modulate mouse embryonic stem cell-derived neural progenitor differentiation via MAP kinases. J Neurochem 112:1431–141

Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T, Catterall WA (1996) Modulation of Ca2+ channels by G-protein beta gamma subunits. Nature 380:258–262

Ippolito DL, Temkin PA, Rogalski SL, Chavkin C (2002) N-terminal tyrosine residues within the potassium channel Kir3 modulate GTPase activity of Galphai. J Biol Chem 277:32692–32696

Ippolito DL, Xu M, Bruchas MR, Wickman K, Chavkin C (2005) Tyrosine phosphorylation of K (ir)3.1 in spinal cord is induced by acute inflammation, chronic neuropathic pain, and behavioral stress. J Biol Chem 280:41683–41693

Land BB, Bruchas MR, Lemos JC, Xu M, Melief EJ, Chavkin C (2008) The dysphoric component of stress is encoded by activation of the dynorphin kappa-opioid system. J Neurosci 28:407–414

Lemos JC, Roth CA, Messinger DI, Gill HK, Phillips PEM, Chavkin C (2012) Repeated stress exposure dysregulates kappa opioid receptor signaling in the dorsal raphe through a p38α MAPK dependent mechanism. J Neurosci 32:12325–12336

Li J, Li JG, Chen C, Zhang F, Liu-Chen LY (2002) Molecular basis of differences in (-)(trans)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidiny)-cyclohexyl]benzeneacetamide-induced desensitization and phosphorylation between human and rat kappa-opioid receptors expressed in Chinese hamster ovary cells. Mol Pharmacol 61:73–84

Luttrell LM, Ferguson SS, Daaka Y, Miller WE, Maudsley S, Della Rocca GJ, Lin F, Kawakatsu H, Owada K, Luttrell DK, Caron MG, Lefkowitz RJ (1999) Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. Science 283:655–661

McDonald PH, Chow CW, Miller WE, Laporte SA, Field ME, Lin FT, Davis RJ, Lefkowitz RJ (2000) Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. Science 290:1574–1577

McLaughlin JP, Xu M, Mackie K, Chavkin C (2003) Phosphorylation of a carboxy-terminal serine within the kappa opioid receptor produces desensitization and internalization. J Biol Chem 278:34631–34640

McLaughlin JP, Land BB, Li S, Pintar JE, Chavkin C (2006) Prior activation of kappa opioid receptors by U50,488 mimics repeated forced swim stress to potentiate cocaine place preference conditioning. Neuropsychopharmacology 31:787–794

McLennan GP, Kiss A, Miyatake M, Belcheva MM, Chambers KT, Pozek JJ, Mohabbat Y, Moyer RA, Bohn LM, Coscia CJ (2008) Kappa opioids promote the proliferation of astrocytes via Gbetagamma and beta-arrestin 2-dependent MAPK-mediated pathways. J Neurochem 107:1753–1765

Melief EJ, Miyatake M, Bruchas MR, Chavkin C (2010) Ligand-directed Jun kinase activation disrupts opioid receptor signaling. Proc Natl Acad Sci USA 107:11608–11613

Miller WE, Houtz DA, Nelson CD, Kolattukudy PE, Lefkowitz RJ (2003) G-protein-coupled receptor (GPCR) kinase phosphorylation and beta-arrestin recruitment regulate the constitutive signaling activity of the human cytomegalovirus US28 GPCR. J Biol Chem 278:21663–21671

Morris GE, Nelson CP, Brighton PJ, Standen NB, Challiss RA, Willets JM (2012) Arrestins 2 and 3 differentially regulate ETA and P2Y2 receptor-mediated cell signaling and migration in arterial smooth muscle. Am J Physiol Cell Physiol 302:C723–34

Pearson G, Robinson F, Beers GT, Xu BE, Karandikar M, Berman K, Cobb MH (2001) Mitogen-activated protein (MAP) kinase pathways. Regulation and physiological functions. Endocr Rev 22:153–183

Pfeiffer A, Brantl V, Herz A, Emrich HM (1986) Psychotomimesis mediated by kappa opiate receptors. Science 233:774–776

Rives ML, Rossillo M, Liu-Chen LY, Javitch JA (2012) 6'-Guanidinonaltrindole (6'-GNTI) is a G protein-biased κ-opioid receptor agonist that inhibits arrestin recruitment. J Biol Chem 287:27050–27054

Samuvel DJ, Jayanthi LD, Bhat NR, Ramamoorthy S (2005) A role for p38 mitogen-activated protein kinase in the regulation of the serotonin transporter: evidence for distinct cellular mechanisms involved in transporter surface expression. J Neurosci 25:29–41

Schattauer SS, Miyatake M, Shankar H, Zietz C, Levin JR, Liu-Chen LY, Gurevich VV, Rieder MJ, Chavkin C (2012) Ligand directed signaling differences between rodent and human kappa opioid receptors. J Biol Chem 287:41595–41607

Schindler AG, Messinger DI, Smith JS, Shankar H, Gustin RM, Schattauer SS, Lemos JC, Chavkin NW, Hagan CE, Neumaier JN, Chavkin C (2012) Stress produces aversion and potentiates cocaine reward by releasing endogenous dynorphins in the ventral striatum to locally stimulate serotonin reuptake. J Neurosci 32:17582–17596

Shippenberg TS, Herz A (1986) Differential effects of mu and kappa opioid systems on motivational processes. NIDA Res Monogr 75:563–66

Simmons ML, Chavkin C (1996) Endogenous opioid regulation of hippocampal function. Int Rev Neurobiol 39:145–196

Sun Y, Cheng Z, Ma L, Pei G (2002) Beta-arrestin2 is critically involved in CXCR4-mediated chemotaxis, and this is mediated by its enhancement of p38 MAPK activation. J Biol Chem 277:49212–49219

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton PM, Miller KJ, Spedding M, Mailman RB (2007) Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 320:1–13

Violin JD, DeWire SM, Yamashita D, Rominger DH, Nguyen L, Schiller K, Whalen EJ, Gowen M, Lark MW (2010) Selectively engaging beta-arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance. J Pharmacol Exp Ther 335:572–579

Wang Z, Gardell LR, Ossipov MH, Vanderah TW, Brennan MB, Hochgeschwender U, Hruby VJ, Malan TP Jr, Lai J, Porreca F (2001) Pronociceptive actions of dynorphin maintain chronic neuropathic pain. J Neurosci 21:1779–1786

Werz MA, Macdonald RL (1984) Dynorphin reduces voltage-dependent calcium conductance of mouse dorsal root ganglion neurons. Neuropeptides 5:253–256

Xu M, Petraschka M, McLaughlin JP, Westenbroek R, Caron MG, Lefkowitz RJ, Czyzyk TA, Pintar JE, Chavkin C (2004) Neuropathic pain activates the endogenous kappa opioid system in mouse spinal cord and induces opioid receptor tolerance. J Neurosci 24:4576–4584

Xu M, Bruchas MR, Ippolito DL, Gendron L, Chavkin C (2007) Sciatic nerve ligation-induced proliferation of spinal cord astrocytes is mediated by kappa opioid activation of p38 Mitogen-Activated Protein Kinase. J Neurosci 27:2570–2581

Yang X, Zhou G, Ren T, Li H, Zhang Y, Yin D, Qian H, Li Q (2012) β-Arrestin prevents cell apoptosis through pro-apoptotic ERK1/2 and p38 MAPKs and anti-apoptotic Akt pathways. Apoptosis 17:1019–1026

Zhu CB, Hewlett WA, Feoktistov I, Biaggioni I, Blakely RD (2004) Adenosine receptor, protein kinase G, and p38 mitogen-activated protein kinase-dependent up-regulation of serotonin transporters involves both transporter trafficking and activation. Mol Pharmacol 65:1462–1474

Zhu CB, Carneiro AM, Dostmann WR, Hewlett WA, Blakely RD (2005) p38 MAPK activation elevates serotonin transport activity via a trafficking-independent, protein phosphatase 2A-dependent process. J Biol Chem 280:15649–15658