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Review

Cross-talk of opioid peptide receptor and β-adrenergic receptor signalling in the heart

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Abstract

Opioid peptide receptor (OPR) and β-adrenergic receptor (β-AR) are well-established members of G-protein-coupled receptor (GPCR) superfamily and are involved in regulating cardiac contractility, energy metabolism, myocyte survival or death. OPRs are typical G_i/G_o-coupled receptors and activated by opioid peptides derived from the endorphin, dynorphin and enkephalin families, whereas β-AR stimulated by catecholamines is the model system for G_s-coupled receptors. While it is widely accepted that β-AR stimulation serves as the most powerful means to increase cardiac output in response to stress or exercise, we have only begun to appreciate functional roles of OPR stimulation in regulating cardiovascular performance. Cardiovascular regulatory effects of endogenous opioids were initially considered to originate from the central nervous system and involved the pre-synaptic co-release of norepinephrine with enkephalin from sympathetic neuronal terminals in the heart. However, opioid peptides of myocardial origin have been shown to play important roles in local regulation of the heart. Notably, OPR stimulation not only inhibits cardiac excitation–contraction coupling, but also protects the heart against hypoxic and ischemic injury via activation of G_i-mediated signalling pathways. Further, OPRs functionally and physically cross-talk with β-ARs via multiple hierarchical mechanisms, including heterodimerization of these receptors, counterbalance of functional opposing G protein signalling, and interface at downstream signalling events. As a result, the β-AR-mediated positive inotropic effect and increase in cAMP are markedly attenuated by OPR activation in isolated cardiomyocytes as well as sympathectomized intact rat hearts. This brief review will focus on the interaction between β-AR and OPR and its potential physiological and pathophysiological relevance in the heart.

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1. Introduction

The Human Genome Project has demonstrated that the family of G-protein-coupled receptors (GPCRs) is the largest and most diverse gene family in the human genome [1]. The GPCR superfamily is involved in the transduction of stimulatory or inhibitory signals in response to a wide array of stimuli, and has also long been considered as a most important therapeutic target. Increasing evidence has shown that members of GPCR superfamily that couple to different classes of G proteins are co-expressed in numerous tissues and interact with each other at multiple levels, including receptor, G protein, or downstream signalling pathways, thus altering signalling and trafficking properties of these receptors. In the heart, β-adrenergic receptors (β-ARs) and opioid peptide receptors (OPRs) are co-expressed, and are coupled to functionally opposite G protein families, G_s and $G_{i/o}$, respectively. Emerging evidence suggests that β_2-AR and OPRs can activate more than one G protein family, as manifested by dual coupling of β_2-AR to G_s and

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G_i and OPR-δ to G_i and G_q. The "cross-talk" between OPRs with β-ARs not only antagonizes β-AR-mediated positive inotropic effect, but also alters these receptors' trafficking and signalling characteristics. In this brief review, we intend to highlight the key features of opioid peptides and OPRs in the heart and their interactions with β-AR signalling.

2. Endogenous opioid peptides: from brain to heart

The pentapeptides methionine-enkephalin and leucine-enkephalin were first discovered in the brain and adrenal gland [2,3]. Shortly after, enkephalin-containing peptides and OPRs were identified throughout the central and peripheral nervous system, including the afferent neurons terminating in the heart [4,5]. Three gene products, pro-opiomelanocortin, prodynorphin, and proenkephalin, are the precursors for endorphins, dynorphins, and enkephalins, respectively [6,7]. The neural signalling of opioid peptides has been well characterized in multiple roles, including bradycardia, tachycardia, hypertension and hypotension [8–12]; however, this has overshadowed the functional roles of endogenous opioids of cardiac origin. Over the past decade, accumulating evidence demonstrates that both proenkephalin and prodynorphin and their final products are expressed and produced directly by cardiomyocytes from mammalian species, including rat, guinea pig, and dog (for review, see Ref. [13]), although opioid peptides are also produced and released in the central nervous system and from peripheral neuronal terminals in the heart where they are co-released with catecholamines [14].

Specifically, a large proportion of enkephalins in the heart appear to be produced by cardiomyocytes [15]. Expression of the proenkephalin gene results in a 31-kDa polypeptide precursor that after post-translational processing yields methionine- and leucine-enkephalin, the heptapeptide methionine-enkephalin-Arg6-Phe7 (MEAP), the octapeptide methionine-enkephalin-Arg6-Gly7-Leu8, and several larger peptides, such as peptides B, E, F, I, and BAM 20P [7]. Proenkephalin mRNA is highly expressed in adult rat heart, particularly in the left ventricle [16]. Although the expression of cardiac proenkephalin mRNA is higher than that in brain [17], the abundance of extracted proenkephalin-derived peptides are much lower in the heart [18]. Interestingly, 95% of enkephalins recovered from rat cardiac ventricle are concentrated in precursor and large intermediate peptides such as proenkephalin (20%), Peptide B (43%), and MEAP (32%) rather than in the smaller end products [19]. Whilst the function of these larger precursor proteins is unclear, they might serve as a ready reservoir or precursor for cleavage into the smaller OPR-active peptides, thereby resulting in augmented release under certain pathophysiological conditions. For instance, leucine-enkephalin release into the coronary sinus effluent in rats is significantly increased in response to ischemia–reperfusion injury [20].

In addition to enkephalins, cardiomyocytes also synthesize and secrete dynorphin B, a biologically active end product of the prodynorphin gene [21], which binds specifically to κ-OPR [22] (see below). It is noteworthy that there is an intrinsic *autocrine loop* regulation of the prodynorphin gene expression. Specifically, dynorphin B, the end product of the prodynorphin gene, stimulates κ-OPR and serves as a positive feedback to augment the prodynorphin gene expression via a mechanism involving translocation of PKC-α into nuclei and subsequent activation of nuclear PKC-δ and -ε [23]. Multiple lines of evidence reveal that prodynorphin gene expression and dynorphin B abundance are markedly increased in cardiomyopathic hearts from Syrian hamsters of the BIO14.6 strain, perhaps largely due to cardiomyopathy-associated abnormalities in Ca^{2+} handling and enhanced activation of PKC [24,25]. Together, these studies strongly support the perception that the heart is a complex endocrine organ, and that myocardial function might be particularly affected by opioid peptides in an autocrine or paracrine manner.

3. Opioid peptide receptors and modulation of cardiac excitation–contraction coupling

Three genetically and pharmacologically distinct OPR subtypes, μ, κ, and δ, have been identified [26–30]. Although these closely related OPR subtypes share about 50% amino acid sequence homology and some functional similarities [31], they differ in their ligand binding and tissue distribution. Specifically, δ-OPRs have the highest affinity for enkephalins; κ-OPRs bind preferentially to dynorphins; and μ-OPRs are selectively sensitive to endorphins, including morphine [32]. Further, it has been demonstrated that δ- and κ-OPRs, but not μ-OPRs, are present in adult rat ventricular myocardium [9,33–38]. In contrast, only μ- and κ-OPRs are observed in neonatal hearts [37]. Thus, there might be a development-dependent expression of δ-OPR in the heart. Under certain pathological circumstances, OPR subtype expression can be up- or down-regulated. Moreover, each OPR subtype can be further subdivided. While δ_1- and δ_2-OPR subtypes have been pharmacologically identified [37,39], only one δ-OPR has so far been cloned [26]. Thus, the existence and significance of δ_1- and δ_2-OPR subtypes merits further investigation. In addition, new subtypes for κ- and μ-OPR have also been reported, but they are yet to be identified in heart.

Importantly, activation of δ-OPRs directly modulate systemic vascular resistance in intact organisms. In adult rat ventricular myocytes, OPR stimulation suppresses the L-type Ca^{2+} current [40] and affects sarcoplasmic reticulum (SR) Ca^{2+} depletion [41], resulting in reduced $[Ca^{2+}]_i$ transient and contractility [41]. These effects are reversed by the OPR antagonist, naloxone. Stimulation of the closely related OPR subtype, κ-OPR, also exhibits a robust inhibitory effect on cardiac excitation–contraction coupling. In

adult rat ventricular myocytes, the κ-OPR agonist, dynorphin B, causes a transient positive inotropic effect via augmenting Ca^{2+} release from SR and intracellular alkalinization, and in the steady-state causes a reduction in myocyte contractility due to SR Ca^{2+} depletion [41–45]. The inhibitory effects of both δ-OPR and κ-OPR on cardiac excitation–contraction coupling are likely mediated by multiple G protein signalling pathways such as $G_{i/o}$ and G_q, as evidenced by their sensitivity to pertussis toxin (PTX, a $G_{i/o}$ inhibitor) and activation of phospholipase C/PKC pathway [41–45].

4. Heterodimerization of β-ARs with OPRs

Increasing evidence has shown that GPCRs can form homodimers or heterodimers. Recent studies suggest that OPRs are capable of forming heterodimers with not only other OPR-subtypes [46,47] but also with β-ARs [48]. Fully functional OPR subtypes can form heterodimers with each other, resulting in a unique population of receptors in terms of ligand binding and intracellular signalling. Heterodimerization of OPR subtypes has been proposed to facilitate selectivity for co-release of the numerous endogenous opioid agonists, thus representing a complex but powerful regulatory mechanism. However, the degree of complexity is even greater than first thought following the finding that oligomerization of OPR with β_2-AR can occur to alter receptor trafficking and signal transduction. Specifically, β_2-AR can physically associate with both δ- and κ-OPRs when they are co-expressed in HEK-293 cells [48]. As a result, activation of δ-OPR by etorphine induces β_2-AR internalisation and inhibits β_2-AR-mediated mitogen-activated protein kinase (ERK1/2) activation [48]. It is presently unclear whether β_1-AR forms a heterodimer complex with OPRs.

5. Cross-talk between β-AR and OPR signalling in regulating cardiac contractility

In the heart, both β-ARs and OPRs are present on cardiac myocyte sarcolemma, and OPR agonists are co-released with the endogenous β-AR agonist, norepinephrine, from nerve terminals [4]. Activation of δ-OPRs not only directly modulates cardiac excitation–contraction coupling, as discussed above, but also markedly inhibits β-AR-mediated positive inotropic effects. For example, leucine-enkephalin (10^{-8} M, a physiologically relevant concentration) overtly attenuates the effect of β-AR stimulation by norepinephrine to increase left ventricular systolic pressure in the isolated perfused rat heart [49], and abolishes β-AR-induced increases in the $[Ca^{2+}]_i$ transient and contractility in single rat ventricular myocytes [50]. These anti-adrenergic effects are reversed by the OPR antagonist, naloxone, and are prevented by PTX pretreatment [49,50].

Similarly, activation of κ-OPR with U50,488H also inhibits the effects of β-AR agonist, norepinephrine (NE), to increase $[Ca^{2+}]_i$ transient and contractility in single isolated rat ventricular myocytes [51]. Interestingly, in ischemic rat hearts, the inhibitory effect of κ-OPR stimulation on β-AR signalling is markedly enhanced, thus resulting in a cardiac protection as evidenced by reduced arrhythmia [52]. In contrast, in chronic hypoxic rat hearts [53] or spontaneously hypertensive rat cardiac myocytes [54], κ-OPR-mediated inhibition of β-AR positive inotropic effect is lacking or markedly blunted. The reduced inhibition of β-AR signalling might contribute to ultimate cardiomyopathy and heart failure under those pathological circumstances (for review, see Ref. [55]).

It is noteworthy that there is a difference between β_1- and β_2-AR subtypes with respect to their cross-talk with G_i/G_o-coupled δ-OPRs in adult rat myocardium (see Fig. 1). The δ-OPR agonist, leucine-enkephalin, markedly inhibits β_1-AR-mediated positive inotropy [49,50]. In contrast, it has no effect on β_2-AR-mediated increase in cardiac contractility [49], indicating that δ-OPR signalling selectively interacts with β_1-AR, but not β_2-AR, in regulating myocardial contractility. Similarly, in cultured neonatal rat cardiomyocytes, the β_1-AR-mediated cAMP accumulation and inotropic and lusitropic effects are all blocked by G_i-coupled M_2-muscarinic receptor stimulation with carbachol. However, the β_2-AR-induced cAMP accumulation and the inotropic effect are insensitive to M_2 stimulation by carbachol [56]. Although β_1-AR activation of inotropy has been shown to be blocked by adenosine, endothelin, and angiotensin, it has not yet been determined whether β_2-AR-induced contractile response is also sensitive to activation of these respective G_i/G_o-coupled receptors in the heart.

The exact mechanism underlying the differential interaction of these β-AR subtypes with G_i-coupled receptors remains elusive. In this regard, it is speculated that the differential interaction between these β-AR subtypes and G_i-coupled receptors such as δ-OPR and M_2-muscarinic receptors might be, to some extent, attributed to the differential subcellular localization of these β-AR subtypes. In the absence of agonist stimulation, β_1-ARs are enriched in non-caveolae cell surface membranes, whereas β_2-ARs are predominantly distributed in the caveolae membrane fraction in neonatal cardiomyocytes [57]. It has been shown that unstimulated M_2-muscarinic receptors co-localize with β_1-ARs, but not β_2-ARs, in non-caveolar cell surface membranes in neonatal cardiomyocytes [58]. This may explain, in part, the differential interactions of M_2-receptor with β_1-ARs versus β_2-ARs. A similar cell architectural arrangement might account for the selective interactions of δ-OPRs with β_1-ARs but not β_2-ARs in the heart. Alternatively, a large body of evidence has indicated that β_2-ARs couple to G_i proteins in addition to the classic G_s pathway [59–64]. The additional G_i coupling of β_2-AR might preclude its interaction with other G_i-coupled receptors such as δ-OPR in regulating cardiac contractility. Altogether, multiple hier-

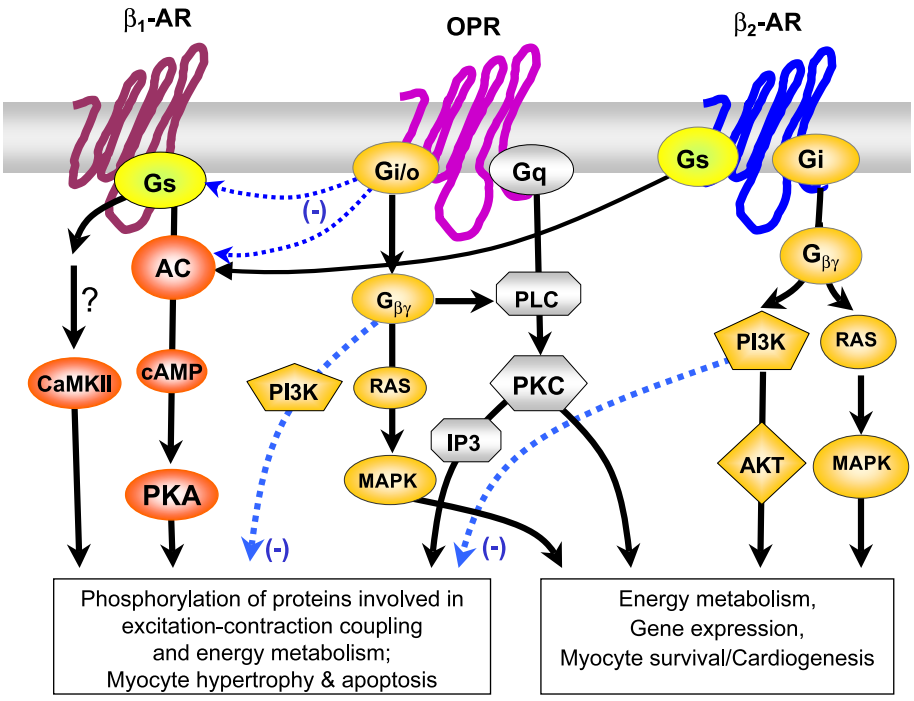

Fig. 1. A simplified scheme of regulatory cross-talk between β-adrenergic receptor (β-AR) subtypes and opioid peptide receptors (OPR) that includes multiple parallel and converging downstream signal transduction pathways. The incidence of receptor heterodimerization is not indicated in the scheme for diagrammatic simplicity. AC: adenylate cyclase; PKA: protein kinase A; PKC: protein kinase C; MAPK: mitogen activated protein kinases; CaMKII: Ca^{2+}/calmodulin-dependent protein kinase II; G_s and G_i proteins: guanidine nucleotide binding proteins with a stimulatory (G_s) and inhibitory ($G_{i/o}$) effect on AC, respectively; Gq: G protein q; $G_{\beta\gamma}$: βγ subunits of G proteins; IP_3: inositol 1,4,5-trisphosphate.

archical mechanisms, particularly their distinct G protein coupling and subcellular localization, may render the subtype-specific β-AR/δ-OPR interaction.

Regarding the possible molecular and cellular mechanisms responsible for the interaction between OPRs and β-ARs, it has been established that OPR stimulation inhibits G_s and adenylate cyclase by activating $G_{i/o}$ signalling pathways, subsequently decreasing cAMP production and PKA activation, leading to a reduction in L-type Ca^{2+} current, depletion of Ca^{2+} from intracellular pools, and reduced contractility in rat and canine hearts [40–45,65–67]. In addition, OPRs are able to directly regulate voltage-gated K^+ channel opening and Ca^{2+} channel closing without the involvement of the second messenger signalling [68–70]. Moreover, the physical interaction between β-AR and OPR might, at least in part, contribute to the inhibitory effect of OPR stimulation on β-AR-mediated positive inotropic and chronotropic effects in the heart.

6. Changes in OPR and β-AR signalling in aging heart

Most but not all studies have provided evidence that opioid peptide gene expression and the abundance of opioid peptides are increased with aging in rat hearts [71–73]. However, it is presently unclear whether the functional effects of OPR stimulation in the heart differ with aging. In contrast, studies over the last three decades have demonstrated that the cardiac response to β-AR stimulation decreases in aging heart [74–77]. Mechanistic studies have been focused on the classic "receptor-G_s-adenylyl cyclase-cAMP-PKA" signalling cascade and found multiple defects in the signalling pathway (for review, see Ref. [74]). The suppression of cardiac response to β-AR stimulation is associated with a significant down-regulation of β-AR density and a decrease in the agonist-stimulated adenylyl cyclase activity. The age-associated up-regulation of OPR signalling might be, in part, responsible for the reduction in β-AR signalling in aging heart, due to the robust antagonistic effects of stimulation of δ-OPR [49,50] or κ-OPR [41–45] on β-AR-mediated positive contractile response.

7. β-AR and OPR signalling in heart failure

Heart failure induced by a variety of causes is associated with elevated levels of circulating catecholamines, plus a concurrent reduction in β-AR density and desensitization of remaining receptors, leading to a markedly blunted βAR contractile response. Specifically, cardiac contractile response to both β_1- and β_2-AR stimulation is markedly diminished in the failing heart. The reduced β-AR inotropic effect is often accompanied by increased amount or activity of G_i proteins [78,79] as well as GPCR kinases (GRKs) [80]

and a selective down-regulation of β_1-AR [81]. Recent studies support the notion that β_1- and β_2-AR activate qualitatively and quantitatively different signalling pathways and may play opposing functional roles in the pathogenesis of heart failure. In particular, sustained β_1AR stimulation not only activates the classic cAMP/PKA signalling pathway but also evokes PKA-independent activation of CaMKII [82] promoting cardiac hypertrophy [83,84] and myocyte apoptosis [82,85–88] and selective β_1AR blockers exhibit beneficial effects in patients with CHF [89], whilst enhanced β_2AR activation appears to be cardiac protective [85,90,91] (see Fig. 1). Thus, it is reasonable to speculate that, in the context of heart failure, the selective down-regulation of β_1AR might represent a complementary cardioprotective mechanism to protect myocytes against apoptosis and slow the progression of cardiomyopathy and contractile dysfunction, whereas the up-regulation of β_2AR signalling could be beneficial due to its contractile support and anti-apoptotic effects. These insights also reveal a potential cell logic for the differential interaction of δ-OPR with β-AR subtypes: β_1-AR, but not β_2-AR, signalling is negated by δ-OPR activation in rat heart [49] (see also Fig. 1).

As discussed earlier, enkephalins negate sympathetic actions on the heart [49–55], in addition to their direct neurally independent negative inotropic effect [40–45]. This might represent a cardioprotective mechanism in the early stage of heart failure to diminish cardiac responsiveness to sympathetic stimulation, thus reducing oxygen demand by limiting work performance. However, exaggerated OPR signalling could contribute to the phenotype of heart failure. For instance, increased levels of Met-enkephalin are correlated with the degree of severity of the disease [92–94]. Further, naloxone is able to improve systemic hemodynamics and myocardial contractile function in a pacing-induced canine heart failure model [95]. Similarly, the heightened opioid peptide activity limits sympathetic activation, whilst inhibition of OPR improves cardiac performance in canine congestive failing hearts [96–97]. The stimulatory effects of the OPR antagonist, naloxone, on the heart could be mediated by an action within the central nervous system [98] as well as a direct inotropic effect [99]. Thus, endogenous enkephalins appear to play an important role in mediating the myocardial depression that occurs in heart failure, and that there may be a role for targeting enkephalins in the clinical therapeutic management of heart failure.

8. Ischemic preconditioning and OPR stimulation

Ischemic preconditioning (IPC) is the phenomenon whereby brief sublethal periods of ischemia protect the heart against a more sustained ischemic event. IPC can be divided into two phases: early preconditioning, occurring immediately after the initial stimulus but with a limited duration of 1–2 h, and late preconditioning, occurring approximately 24 h after the initial stimulus with a duration of almost 72 h [100]. IPC has been shown in both humans [101] and animals [102,103] to reduce infarct size and improve functional recovery after a prolonged period of ischemia.

Considerable evidence points to cardiac mitochondria as contributing an important role in cardioprotection against ischemia–reperfusion injury. The myocardium has a high metabolic rate and is therefore highly dependent on mitochondria for ATP production [104]. Conditions associated with ischemia, prolonged hypoxia and/or deprivation of substrates can ultimately lead to mitochondria-dependent cell death. A key mechanism of action in IPC involves ATP-sensitive potassium channels (K^+_{ATP} channels). Overexpression of recombinant K^+_{ATP} channel subunits or pharmacological stimulation of the channels has been shown to promote cytoprotection [104]. Although initially it was suggested that cell surface K^+_{ATP} channels were responsible for this effect, increasing evidence suggests that *mitochondrial* K^+_{ATP} channels, which are pharmacologically and histochemically distinct from sarcolemmal K^+_{ATP} channels [105–109], are the key channels in this process. Opening of mitochondrial K^+_{ATP} channels after protein kinase C activation and translocation has been shown to be crucial for cardioprotection [110,111].

Multiple lines of evidence suggest that blocking OPR with naloxone or naltrindole can abolish the effects of IPC in humans [112] and rats [113], indicating that δ-OPR activation is involved in IPC. This suggests that δ-OPR stimulation by endogenous enkephalins may play a major role in IPC [114–118] and may have significant impact on cardiac protection and organ preservation for transplantation in the clinical arena. Recent studies have shown that, similar to δ-OPR signalling, κ-OPR stimulation plays an important role in IPC-induced cardiac protection. In fact, κ-OPR is involved in IPC-induced ameliorating effects on arrhythmia as well as infarct, whereas δ-OPR activation is only involved in IPC-mediated anti-arrhythmia in perfused rat hearts [119]. The protective effects of κ-OPR stimulation are dependent on activation of PKC and K^+_{ATP} channels [119].

The cardioprotective signalling pathways linking OPR signalling to mitochondrial K^+_{ATP} channel activation, and other subcellular targets as seen after PKC isomer activation, awaits future investigation. However, a notable recent breakthrough is the demonstration that numerous types of G-protein-coupled receptors (including opioid receptors), when activated elicit cell protection by signalling via PKB/Akt, mTOR/p70s6k, PI3K, PKC, or PKA pathways which converge to ultimately inhibit GSK-3β. The inhibition of GSK-3β impacts the mitochondrial permeability transition pore complex, to limit induction of mitochondrial pore opening and permit survival following cellular stress [120,121]. As strict metabolic regulation and repair/growth mechanisms are crucial requirements for survival, it is likely

that these pathways are also converged or shared, at least in part, with those involved with the adaptive processes active in aged, hypertrophic or failing hearts.

9. Involvement of OPR stimulation in cardiogenesis

Emerging evidence indicates that the prodynorphin gene and its product, dynorphin B, play an important role in cardiogenesis. It has been shown that P19 embryonal pluripotent stem cells are able to express the prodynorphin gene and produce dynorphin B, and that stimulation of κ-OPRs with dynorphin enables these cells to express GATA-4 and Nkx-2.5 genes, key transcription factor-encoding genes essential for cardiogenesis and expression of α-myosin heavy chain and myosin light chain-2 V genes, two markers of cardiac differentiation [122]. More recently, studies from the same laboratory have further elucidated that in GTR1 embryonic stem cells, stimulation of κ-OPRs promotes cardiogenesis which is associated with activation of PKC-δ and -ε mainly at the nuclear level and translocation of PKC-α, -β_1, and -β_2 isozymes from cytosol to nucleus, suggesting PKC signalling constitutes the major molecular and cellular mechanism underlying OPR-mediated cardiogenesis in GTR1 embryonic stem cells [123,124]. This notion is further supported by the fact that inhibition of OPR by receptor antagonist reduces cardiomyocyte yield and that PKC inhibitors block the expression of cardiogenic genes and dynorphin B production in the embryonic stem cells and prevent their in vitro differentiation into beating cardiomyocytes [123,124]. Taken together, these recent studies indicate that OPR stimulation regulates cardiogenesis via an autocrine loop signalling mechanism sequentially involving κ-OPR activation by the endogenous agonist, dynorphin B, an increase in PKC activity in the nucleus and expression of cardiac progenitor genes.

In addition, emerging evidence suggests that OPR stimulation serves as a negative growth regulator in renewing and regenerating epithelia, and that disrupting OPR signalling promotes basal cell proliferation [125]. Furthermore, δ-OPR stimulation can abolish β_2-AR-mediated cell proliferative effects, indicating that a cross-talk occurs between δ-OR and β_2-AR signalling in regulating cell proliferation rate [126].

10. Conclusion

Opioid peptides, in particular, enkephalins, have been investigated extensively in the central nervous system. Recently, a novel role of opioids as modulators of cardiac function has emerged. Stimulation of δ- and κ-OPR not only exhibits a cardioprotective effect against ischemic and hypoxic injury and directly suppresses cardiac excitation–contraction coupling, but also markedly negates β-AR-mediated positive inotropic effects. The cross-talk between the two functionally opposite GPCR families might have important physiological and pathological relevance in cardiac aging, heart failure, and cardiac responses to ischemic stress. The exact mechanisms underlying the interaction of these two GPCR systems merit further investigation.

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