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Opioid-induced Cardioprotection

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Abstract

Ischemic heart disease and myocardial infarction continue to be leading causes of cardiovascular morbidity and mortality. Activation of opioid, adenosine, bradykinin, adrenergic and other G-protein coupled receptors have been found to be cardioprotective. κ - and/or δ -opioid receptor activation is involved in direct myocardial protection, while the role of μ -opioid receptors seems less clear. In addition, differential affinities to the three opioid-receptor subtypes by various agonists and cross-talk among different G-protein coupled receptors render conclusions regarding opioid-mediated cardioprotection challenging. The present review will focus on the protective effects of endogenously released opioid peptides as well as exogenously administered opioids such as morphine, fentanyl, remifentanyl, butorphanol, and methadone against myocardial ischemia/reperfusion injury. Receptor heterodimerization and cross-talk as well as interactions with other cardioprotective techniques will be discussed. Implications for opioid-induced cardioprotection in humans and for future drug development to improve myocardial salvage will be provided.

1. Introduction

Ischemic heart disease continues to be the most significant cause of morbidity and mortality in the Western world [1]. While prevention aims to reduce risk factors for myocardial infarction [2], other strategies can ameliorate myocardial damage when applied before, during, or shortly after myocardial ischemia and reperfusion (IR) [3, 4]. One of the most efficacious methods among these is ischemic preconditioning (IPC), discovered by Murry

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and colleagues in 1986 [3]. It involves several brief periods of coronary artery occlusion and reperfusion prior to prolonged ischemia to attenuate ATP depletion and tissue damage. Subsequently, IPC was found to be blocked by opioid receptor antagonists [5] and mimicked by the opioid receptor agonist morphine [6], suggesting that a viable strategy to reduce myocardial injury could involve activation of opioid receptor signaling pathways. Opioid receptors are selectively activated by endogenous opioid peptides or exogenous agents (Table 1) commonly given for analgesia which can effectively reduce myocardial ischemia/reperfusion (IR) injury in both animal models and in humans. More recently, μ -receptor agonists have been reported to reduce myocardial infarct size *in vivo* or in rat isolated hearts [7, 8], although this action may be dependent on activation of extra-cardiac opioid receptors, such as are present in the central nervous system (CNS) [7].

In the following review, we will provide an overview of mechanisms of opioid-induced protection against myocardial IR injury, as observed in cells, tissues and whole organs and in different species including humans, and provide an outlook on future directions and drug development.

2. Opioid Receptors

Endogenous and exogenous opioid agonists exert their pharmacological and physiological effects through binding to specific opioid receptors. The opioid receptor family consists of three major single-gene derived classes: μ , κ , and δ (table 1) [9]. All three opioid receptors are seven-transmembrane spanning proteins that couple to inhibitory G-proteins. The structure and function of opioid receptors are well described in a review by Minami and Satoh [9]. In addition to these three subtypes, a novel kind of “opioid receptor-like orphan receptor” (ORL1) was discovered and cloned almost 20 years ago [10]. The ORL1 G-protein coupled receptor [11] is highly homologous to the classical opioid receptors, but does not bind well to opioid ligands [10]. Soon after the discovery of ORL1, nociceptin (also called orphanin FQ) was identified as its endogenous ligand.

Interestingly, most studies have found δ - and κ -, but not μ -receptors expressed in cardiomyocytes. In rat isolated cardiomyocytes, Ventura and colleagues reported that both κ - and δ -, but not μ -selective radioligands exhibited a high affinity, suggesting the presence of δ - and κ -receptors in cardiomyocytes [12]. The κ -binding site, more specifically κ_1 , was reported in the crude membrane preparation of a rat heart homogenate [13], and both κ - and δ -, but not μ -receptors, have been identified in rat atrial and ventricular tissue [14]. Furthermore, opioid peptides were shown to have marked effects on cardiac muscle function in rat ventricular cardiomyocytes mediated by κ - and δ -receptor, but not μ -receptor stimulation [15]. In peripheral tissues of the rat κ -, δ - and μ -opioid receptors have been demonstrated to be widely expressed in several tissues including the small intestine, large intestine, adrenals, kidneys, lung, spleen, testis, ovaries and uterus using reverse transcriptase polymerase chain reaction (RT-PCR) and Southern blotting [16]. In contrast, predominantly δ -transcripts, with no μ -receptor and a weak signal for the κ -receptor were found in cardiac tissue [16]. Similarly, κ - and δ -receptors and their ligand precursors were found to be expressed in human atrial tissue of patients in sinus rhythm or persistent atrial fibrillation [17]. Interestingly, κ -opioid ligand precursors as well as receptor mRNA were

quantitatively decreased in patients with atrial fibrillation compared to those in sinus rhythm, whereas the expression of corresponding δ -receptors was unchanged [17]. All of the above suggests that κ - and δ -receptors, but not μ -receptors are expressed in cardiac tissues.

Conversely, two studies reported evidence of μ -receptors in cardiac tissues. Using RNA isolation and RT-PCR analysis in human atria and ventricular tissue, Bell and colleagues demonstrated the presence of δ - and μ - receptor in human ventricular tissue at a copy number similar to human atrial tissue, but at a higher copy number than κ -opioid receptors [18]. However, origination of the κ -PCR product from neuronal tissue within the myocardium could not be excluded. In 2005, Head and colleagues provided additional evidence consistent with the idea that functional μ -receptors were expressed in cardiomyocytes of adult rat hearts by using four different techniques (PCR, Western blotting, immunofluorescence microscopy, and assay of cAMP generation) [19]. Very recently, Peng and colleagues developed a quantitative real-time RT-PCR assay to quantify μ -, κ -, and δ -receptor mRNA in human tissue [20]. Although mRNA expression of all three opioid receptors was high in most of the CNS area examined (1×10^5 to 1×10^7 copies/ μ g), κ - and δ -receptor mRNA expression was detected at very low levels in the heart (6,363 and 3,810 copies/ μ g, respectively) and μ -receptor mRNA was absent. Taken together, it cannot be excluded that μ -receptors are present in the heart, but their detection may be challenging because of low copy number, so that further studies will be required to definitively settle this issue.

3. Endogenous Opioids in the Heart

Cardiac myocytes are capable of synthesis, storage, and release of opioid receptor peptides [21]. The endogenous opioid peptide family consists of endorphin, dynorphin, and enkephalin, and their associated μ -, κ - and δ -opioid receptors, respectively (table 1). Endogenous opioid peptides contribute to ischemic tolerance by both pre- and postsynaptic mechanisms. Opioid receptor agonists have been shown to act via G_i -linked pathways that alter myocardial ion channel activity and the intracellular activity of protein kinases [22]. Endogenous opioid peptides including both met-enkephalin and dynorphin B significantly increased in quantity after myocardial ischemia as detected by radioimmunoassay [23]. Met- and leu-enkephalin reduced the incidence of cell death in isolated adult rabbit cardiomyocytes subjected to hypoxia, and this action did not occur with administration of β -endorphins that bind primarily to μ -receptors [24]. These findings indicate that enkephalins that bind to δ -receptors and dynorphins that bind to κ -receptors play an important role in opioid-induced improvement of functional recovery and limitation of cell death.

In 1986, Murry and colleagues discovered that brief periods of repeated IR protected the myocardium from damage that occurred during a subsequent more prolonged IR and called this phenomenon ischemic preconditioning (IPC) [3]. Ten years after this landmark discovery, Schultz and colleagues reported that endogenous opioids played an important role in IPC and significantly limited myocardial infarct size after IR in an *in vivo* rat model. However, naloxone, a non-selective opioid antagonist, given before or immediately after IPC abolished this protective effect [5]. The receptor-active stereoisomer (–)naloxone, but not the receptor-inactive stereoisomer (+)naloxone, blocked IPC in an *in vivo* rabbit model

[25]. IPC-induced attenuation of cardiomyocytes apoptosis in rabbits was also abolished by naltrindole, a selective δ -antagonist [26]. These results demonstrate a crucial role for endogenous opioid peptides to activate opioid receptors during endogenous cardioprotection.

4. The Role of Opioid Receptors in Opioid-Induced Cardioprotection

4.1. δ -Receptor

Not surprisingly – given the existence of δ -receptors in the heart described above – various studies support the evidence that activation of δ -receptors can reduce myocardial IR injury. Exogenous administration of selective δ -opioid agonists has been reported to be cardioprotective when given acutely before ischemia, or immediately before reperfusion. For example, the selective δ -opioid agonist BW373U86 and the non-selective opioid agonist morphine reduced infarct size compared to vehicle when administered 10 min before ischemia or 5 min before reperfusion [27]. Similarly, two selective δ -opioid agonists, TAN-67 and BW373U86, produced significant reductions in infarct size similar to that of IPC in dogs [28]. In rabbits, morphine-induced attenuation of cardiomyocyte apoptosis was abolished by naltrindole, a selective δ -antagonist [26]. Fentanyl isothiocyanate, which binds selectively and irreversibly to the δ -receptor, was reported to reduce infarct size equally when given prior to ischemia or prior to reperfusion in an *in-vivo* rat model, and this protection, as assessed by infarct size reduction, was extended to 10 sec after reperfusion [29]. Moreover, in human atrial trabeculae, the δ -receptor agonist D-Ala2-Leu-enkephalin (DADLE) mimicked the effects of IPC [18]. Schultz and colleagues further demonstrated that IPC-induced reduction of myocardial infarct size in rats *in vivo* was mediated by the δ_1 -receptor, but not the δ_2 -, μ -, or κ -receptor because the selective δ_1 -antagonist BNTX (7-benzylidenenaltrexone), but not the δ_2 -antagonist naltriben, the irreversible μ -antagonist β -funaltrexamine or the selective κ -antagonist nor-BNI (norbinaltorphimine), abolished IPC [30]. These results suggest that the stimulation of δ -receptors with selective agonists can mimic the cardioprotective effects of IPC.

4.2. κ -Receptor

Although clear evidence indicates that activation of the δ -receptor reduces myocardial infarct size in many species including human, the role of κ -opioid receptors in cardioprotection is more controversial. Several studies reported that exogenous activation of the κ -receptor induced either anti-infarct or anti-stunning effects [31–33]. U50,488, a κ -receptor agonist, reduced myocardial infarct size compared to control experiments via PI3K and mitochondrial ATP-sensitive potassium channels (mK_{ATP}) when given prior to, but not after reperfusion [31]. κ -receptor activation may produce anti-arrhythmic effects [34]. Wang and colleagues showed that κ -receptors mediated the beneficial effects of IPC on infarct size and arrhythmias in isolated rat hearts subjected to IR, whereas δ -receptors were responsible for modulation of infarct size only [35]. Conversely, specific activation of the κ -receptor before ischemia has been reported to paradoxically increase myocardial infarct size [36] and arrhythmias [37]. Further studies are warranted to address the specific role of κ -receptor activation with regards to timing of agonist administration and amelioration of arrhythmias.

4.3. μ -Receptor

As described in section 2, Opioid Receptors, earlier studies questioned the presence of μ -receptors in cardiac tissue. Enkephalin or dynorphin that bind to δ - or κ -receptors, respectively, have been implicated in IPC, endogenous opioid-induced preconditioning, and/or reaction to myocardial ischemia. In contrast, β -endorphin that binds to μ -receptors does not appear to be similarly involved (section 3, Endogenous Opioids in the Heart). However, remifentanyl which has a high degree of μ -receptor selectivity with a lower affinity for the δ - and κ -receptor [38] mimicked IPC (section 5, Cardioprotection by Clinically Used Opioids). Recently, Gross and colleagues have demonstrated that Eribis peptide (EP) 94, a novel enkephalin derivative that binds with high potency to δ - and μ -receptors, produced an acute reduction in myocardial infarct size and that this action was abolished by the μ -antagonist CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂), but not the δ -antagonists naltrindole or BNTX, or the κ -antagonist nor-BNI [39]. They further tested the hypothesis that EP 94's protective effect may be mediated by acting in the CNS rather than the heart directly (also see section 5.1, Morphine). The non-selective opioid antagonist naloxone hydrochloride which penetrates the blood-brain barrier abolished the acute reduction in myocardial infarct size induced by EP 94, whereas naloxone methiodide that is unable to cross the blood-brain barrier did not. These results indicate that EP 94 may have acted through central μ -receptors to achieve cardioprotection, possibly via descending neural pathways increasing parasympathetic and/or decreasing sympathetic output [39].

4.4. Cross-Talk between Receptors

Several receptor ligands have been associated with cardioprotective effects, including adenosine, bradykinin, opioids, erythropoietin, adrenergic and muscarinic agonists [40]. A "multiple trigger theory" has been suggested in which the activation of any member of the G-protein coupled receptor family converges on a common cardioprotective target, thought to be protein kinase C (PKC) [41]. Some well characterized examples of receptor cross-talk may have been the result of direct heterodimerization between G-protein coupled receptors [42]. For example, cardioprotection mediated by either morphine or the selective adenosine A₁-receptor agonist 2-chloro-cyclopentyladenosine (CCPA) were attenuated by a selective A₁-antagonist as well as a selective δ ₁-antagonist [43]. In Langendorff-prepared rat hearts, fentanyl enhanced the recovery of cardiac mechanical function after ischemia, and this action was attenuated by naloxone or a selective A₁-antagonist [44]. Similarly, inhibitory cross-talk appears to exist between δ -opioid and β ₁-adrenergic receptors resulting in attenuation of the myocardial responses to stress. In isolated rat hearts, a marked anti-adrenergic effect of the δ -receptor agonist leu-enkephalin, due to its postsynaptic action on the β ₁-adrenergic receptor was observed; this likely occurred via a pertussis toxin (PTX)-sensitive G-protein involved in adenylyl cyclase inhibition [45]. Likewise, cross-talk between κ -opioid and β -adrenergic receptors has been reported [46, 47]. The effects of norepinephrine on both contraction and [Ca²⁺]_i transients measured in single rat ventricular myocytes were attenuated in a dose-dependent manner by the κ -agonist U50,488H. This suggested that κ -receptor stimulation produced inhibitory actions on β -adrenergic receptor stimulation in the heart. U50,488H also inhibited the stimulatory effects of the β -adrenoceptor agonist isoprenaline on [Ca²⁺]_i transients and cAMP accumulation in ventricular myocytes, and this cross-talk was attenuated in myocytes from chronically

hypoxic, but not normoxic rats [46, 47]. This action was attributed to impaired G_s -protein and adenylyl cyclase responses to κ -receptor activation and to desensitization of the receptor.

Using autoradiographic mapping of A_1 - and A_{2A} -adenosine receptors and adenosine transporters in the brain and spinal cord of μ -opioid receptor knockout (KO) mice small site-specific reductions in A_1 -receptor and transporter binding were observed compared to wildtype mice [48]. These results suggested functional interactions between μ -opioid and A_1 -adenosine receptors as well as adenosine transporters in the brain, but not in the spinal cord.

Co-expression of μ - and δ -receptors in heterologous cells was shown to result in the isolation of newly formed ~150 kDa receptor heterodimers. Treatment with δ -selective ligands increased μ -agonist binding and vice versa, but only in cells co-expressing both receptors. Thus, heterodimerization may explain the molecular basis for cross-modulation and enhanced potency of μ - and δ -receptor agonists [49]. Devi's laboratory also demonstrated in HEK293, Neuro2A, and SKNSH cells that the association with RTP4, a Golgi chaperone, protects heterodimers of μ and δ receptors from ubiquitination and degradation and results in increased surface heterodimer levels [50]. This study indicates that in the absence of RTP4, μ - δ heterodimers are largely retained in the Golgi apparatus and eventually routed to degradation pathways, while in the presence of RTP4, the heterodimers are stabilized and routed to the membrane, leading to a reduced population of receptor homodimers. This may lead to changes in the extent of receptor signaling and represent a critical factor influencing the action of exogenous and endogenous opioid ligands.

Recently, the concept of receptor heteromers is becoming generally accepted: receptors of the same and/or of different gene families can combine to generate dimers and possibly higher-order units with unique biochemical and functional characteristics. Opioid receptors are part of the studied receptors. For further review on the topic of receptor heteromers please refer to reference [51]. Using newly generated μ - δ heteromer-selective antibodies, it could be shown that chronic, but not acute, treatment with morphine increased μ - δ heteromer abundance in regions of the brain that are involved in pain perception [52]. Although limited to the neuronal systems at this point, these findings may have future implications for other organ systems, including the heart.

Complex interactions exist between opioids and cytokines within the nervous and immune systems. For example, interleukin-2 (IL-2) is generated from activated helper T lymphocytes and stimulates proliferation and effector functions in various cells of the immune system. In hamster papillary muscle, IL-2 was reported to have negative inotropic effects that were partially mediated by NO [53].

Cao and colleagues examined the effects and responsible mechanisms of IL-2 on contraction and $[Ca^{2+}]_i$ transients in the isolated ventricular myocytes [54]. IL-2-induced decreased both contraction and $[Ca^{2+}]_i$ transients that were abolished by pretreatment with naloxone, or with a specific κ -antagonist, nor-BNI, but not by pretreatment with the specific δ -antagonist naltrindole [54]. The effect of IL-2 was also abolished by pretreatment with PTX suggesting

that IL-2's effects on contraction and $[Ca^{2+}]_i$ were mediated by the cardiac κ -receptor, and that the signal transduction pathway included a PTX-sensitive G-protein. Additionally, IL-2 decreased infarct size and LDH release in rat isolated hearts subjected to IR. IL-2-induced protection was blocked by nor-BNI, but not naltrindole [55], providing further evidence of an interaction between IL-2 and κ -, but not δ -receptors during cardioprotection.

Cross-talk between opioid receptors and caveolae has also been demonstrated [56–58]. Lipid rafts, a subdomain of the plasma membrane, are defined by their enrichment in cholesterol and glycosphingolipids, especially in the outer leaflet of the lipid bilayer [56]. Caveolae were identified microscopically in the 1950s as approximately 100 nm invaginations of the plasma membrane that have a lipid composition similar to that of lipid rafts but in addition, possess an organelle-specific structural protein, caveolin [57]. The three subtypes of caveolins (caveolin-1, -2, and -3) are known to have a similar overall molecular organization, but differ in their primary sequence and expression in particular tissues [59]. Caveolin-3, for example, is uniquely expressed in skeletal and cardiac myocytes. As many physiologically important G-protein coupled receptors, including endothelin, muscarinic, bradykinin, β -adrenergic, adenosine A_1 , dopamine, and μ -opioid receptors have been shown to localize in lipid raft/caveolae microdomains before or after activation [56, 58], it is conceivable that G-protein coupled receptors functionally depend on caveolae.

Using wild type, caveolin-3 KO and caveolin-3 overexpressing mice, Tsutsumi and colleagues [60] showed that while myocardial infarct size and the release of cardiac troponins were reduced in wild type mice *in vivo* by pretreatment with the δ -selective opioid agonist SNC-121, no protection was observed in caveolin-3 KO mice. In contrast, caveolin-3 overexpressing mice showed innate protection against IR injury which was abolished by naloxone. These findings suggest that opioid-induced preconditioning is dependent on caveolin-3 expression, and conversely, that endogenous protection by caveolin-3 overexpression is opioid-dependent.

4.5. Signaling Pathway

Opioid-induced cardioprotection and IPC have been shown to share common pathways. A large body of evidence has demonstrated that mitochondrial [61, 62] and sarcolemmal K_{ATP} channels [63], reactive oxygen species [43, 64, 65], PKC [66], GSK-3 β [27], mTOR [27], p38 MAPK [67], ERK [67], iNOS [64], JAK/STAT [68] and COX₂ [64] are involved in cardioprotection by opioids (summarized in Figure 1). For more details on signaling pathways, the reader may refer to previous reviews [22, 69, 70].

5. Cardioprotection by Clinically Used Opioids

5.1. Morphine

As discussed in section 3, Endogenous Opioids in the Heart, opioids play a critical role to mediate IPC. Not only did naloxone abolish IPC [5], but morphine mimicked the cardioprotective effect of IPC, and this effect was mediated by K_{ATP} channel activation [6]. Morphine-induced PC was elicited by three 5-min drug infusions (100 μ g/kg IV) interspersed with 5-min drug-free periods before 30 min coronary artery occlusion and 2 hrs reperfusion in an *in vivo* rat model [6]. IPC and morphine PC reduced myocardial infarct

size to a similar degree [6]. Although morphine is commonly classified as a μ -opioid agonist because of its high affinity for the μ -receptor, its concomitant interaction with κ - and δ -receptors in fact makes it a nonspecific opioid agonist [22, 71]. The highly selective δ -antagonist naltrindole completely abolished morphine-induced infarct size reduction, indicating that morphine-induced infarct size reduction is elicited via δ -receptors [72]. Nevertheless, cross-talk between δ - and μ -receptors (for details see section 4.4.) may also be involved [22]. Morphine attenuated neutrophil and endothelial activation in patients with acute myocardial infarction and reduced the amount of adhesion molecules in rat vena cava blood, suggesting that this opioid agonist may produce diverse effects to reduce IR injury [73, 74]. Morphine administration increased the activities of neutrophil endopeptidase 24.11 (enkephalinase), a zinc metalloprotease that hydrolyzes various naturally occurring peptides including met- and leu-enkephalin in patients with acute myocardial infarction, and attenuated shedding of L-selectin and ICAM-1 [73]. These data suggest that morphine-induced inhibition of neutrophil-endothelium activation may produce favorable effects in patients with acute myocardial infarction.

Most investigations have concluded that cardioprotection by opioids does not involve μ -receptor activation [24, 30]. However, smaller doses of morphine administered intrathecally (IT) in rats reduced myocardial infarct size to a similar extent as morphine IV [75]. Interestingly, all morphine-pretreated groups demonstrated significant reductions in infarct size compared to control experiments, and there were no significant differences in infarct size among the morphine-pretreated rats regardless of route of administration or dosage. Another group reported similar findings that any dose of IT morphine from 0.3 to 30 $\mu\text{g/kg}$ reduced myocardial infarct size compared to control experiments, and that the beneficial effect was attenuated by either δ -, μ -, and κ -selective antagonists [76]. These interesting results suggested that the minimal dose at which IT morphine PC occurs is 1/1000th of the necessary IV dose which is of a similar order of magnitude required to modify pain behavior in rats [77]. Activation of all three opioid receptors in the CNS is required to produce a remote PC effect against IR, and this action is mediated by activated calmodulin and downstream release of calcitonin gene-related peptide [78]. In addition, any one of the three used opioid receptor antagonists abolished this effect indicating that the activation of δ -, μ -, and κ -receptors is likely necessary during morphine PC.

Alternatively, IPC-induced acute reduction in myocardial infarct size may be mediated by a peripheral opioid receptor mechanism [79, 80]. For example, regular naloxone, a non-selective opioid receptor antagonist, and its quaternary derivative naloxone methiodide that does not cross the blood-brain barrier, were used to determine the role of central vs peripheral opioid receptors during IPC in an *in vivo* rat model of myocardial IR [79, 80]. Low dose naloxone did not, but high dose naloxone completely blocked IPC. Interestingly, low dose naloxone methiodide blocked IPC only partially, whereas high dose naloxone methiodide completely blocked IPC, indicating that IPC-induced infarct size reduction is mediated by a peripheral opioid receptor mechanism in the intact rat heart. These findings were confirmed two years later in a rabbit *in vivo* and an isolated heart model [79, 80]. IPC was blocked when naloxone methiodide was given 1 min before IPC *in vivo*. While infarct size was intermediate when low dose naloxone hydrochloride was given before IPC in the

isolated heart, IPC was completely blocked by high dose naloxone hydrochloride. Their findings also suggest that IPC involves intra-cardiac opioid receptor activation.

More recently, Lu and colleagues found that morphine PC produced a similar cardioprotective effect as IPC in an *in vivo* rat model. When the quaternary naloxone methiodide was given IV or IT before IPC, however, they found that while both central and peripheral opioid receptors mediated the reduction in infarct size by morphine PC, only peripheral opioid receptors mediated IPC [81]. Taken together, the CNS may serve as an alternative site of action for morphine-induced protection against myocardial infarction.

5.2. Fentanyl

Fentanyl is considered to be selective for μ -receptors, it can also interact with δ - and κ -receptors [82]. Similarly to morphine [72], fentanyl exerted its protective effects against myocardial IR injury via activation of δ -receptors [44, 83]. Fentanyl enhanced recovery of cardiac contractile function in Langendorff rat hearts following 30 min ischemia [44], and this effect was abolished by pretreatment with naloxone, the mK_{ATP} channel blocker 5-hydroxydecanoate, or the adenosine A_1 -antagonist DPCPX. These findings suggest that opioid and A_1 -receptor activation as well as mK_{ATP} channel opening are involved in the mechanism of enhanced recovery of cardiac contractile function induced by fentanyl. Although the involvement of PKC was not specifically addressed, both δ -receptors and A_1 -receptors have been reported to activate PKC via G proteins in myocytes [71, 84], and PKC activation may have been the link between stimulation of opioid receptors and activation of mK_{ATP} channels. Subsequently, fentanyl-induced reduction in infarct size and enhanced recovery of cardiac contractile function was shown to be mediated by PKC and by δ -receptors [83] during rat Langendorff experiments in which protection against myocardial infarction was blocked by the δ -receptor antagonist naltrindole or the PKC inhibitor chelerythrine [83]. These results suggest that fentanyl protects the heart against myocardial IR injury via δ -receptor cross-talk with adenosine A_1 -receptors and through PKC- and mK_{ATP} -linked mechanisms.

5.3. Remifentanyl

Remifentanyl is an ultra-short acting opioid that is rapidly metabolized by nonspecific blood and tissue esterases [85] and, similar to fentanyl, possesses a high affinity for μ -receptors and a lower affinity for δ - and κ -receptors [38]. Remifentanyl afforded similar protection to IPC in rats *in vivo* [7]. Interestingly, decreases in myocardial infarct size produced by remifentanyl were blocked by the opioid receptor antagonists naltrindole (δ), nor-BNI (κ), and CTOP (μ), while IPC was only sensitive to δ - and κ -receptor antagonism. Since it is entirely possible that remifentanyl also acts on μ -receptors outside the myocardium, e.g. through the CNS (also see section 5.1, Morphine) the authors tested this hypothesis by using rat isolated hearts precluding a possible CNS involvement [8]. The remifentanyl-induced reduction of myocardial infarct size after IR was abolished by pretreatment with naltrindole and nor-BNI but not with the μ -selective antagonist CTOP in this study, suggesting that cardiac δ - and κ -, but not μ -receptors mediate myocardial protection by remifentanyl. Furthermore, these investigators demonstrated that the mechanisms responsible for remifentanyl-induced reduction in infarct size involved both PKC activation and mK_{ATP}

channel opening. PKC activation by remifentanyl has also been found in rat hearts *in vivo* [86]. Thus, the evidence suggested that remifentanyl, despite a high affinity for μ -receptors, mimics IPC via cardiac δ - and κ -receptors and partly via extra-cardiac μ -opioid receptors. Nevertheless, unlike the possible involvement of calmodulin and calcitonin gene-related peptide [78] in the case of morphine, it is still unclear how the activation of extra-cardiac opioid receptors by remifentanyl produces myocardial protection. Further studies are required to elucidate detailed mechanisms.

5.4. Butorphanol

The mixed agonist-antagonist butorphanol was found to increase the threshold of IPC in adult open-chest dogs [87]. A single 5-min episode of ischemia followed by 10 min of reperfusion as a PC stimulus was blocked by butorphanol. In contrast, pretreatment with butorphanol did not abolish the reduction in myocardial infarct size induced 2 or 4 cycles of ischemic PC. The exact mechanism of butorphanol to increase the threshold of IPC is still unclear.

5.5. Methadone

Methadone is a potent μ -opioid agonist and N-methyl-D-aspartate receptor antagonist. This synthetic opioid agonist was developed more than 40 years ago [88]. Administration of methadone (0.3 mg/kg) or morphine (0.3 mg/kg) before ischemia reduced myocardial infarct size in rats *in vivo*, while pretreatment with the δ -opioid antagonist naltrindole before methadone or morphine blocked the reduction in myocardial infarct size [89]. Methadone (0.3 mg/kg) reduced myocardial infarct size when given 5 min before reperfusion but not 10 sec after reperfusion. These results suggest that methadone and morphine protect against infarction through δ -receptor mediated effects.

6. Combination of Opioids and Volatile Anesthetics

In 1997, volatile anesthetics were shown to elicit a PC effect to attenuate myocardial IR injury in dogs [90]. Since then, numerous studies have elucidated the signaling mechanisms responsible for this pharmacological approach to cardioprotection. For more details on anesthetic PC, the reader may refer to recent reviews [91–93].

Volatile anesthetics and opioids that trigger cardioprotective mechanisms may produce additive, synergistic or even competitive effects. Several investigations support the contention that volatile anesthetics and opioids produce additive effects. Using the mK_{ATP} channel opener diazoxide, the volatile anesthetic isoflurane, and the delta opioid agonists TAN-67 or BW373U86 alone or in combination in an *in vivo* rat model, opioids and volatile anesthetics were demonstrated to decrease myocardial infarct size through δ -opioid receptors and potentiation of cardiac mK_{ATP} channel opening [94]. In another study, isoflurane (1.0 minimal alveolar concentration [MAC]) and 0.3 mg/kg morphine when given together before IR decreased infarct size to a greater extent than either drug alone; since this effect was abolished by the mK_{ATP} channel antagonist 5-HD and the opioid antagonist naloxone, both opioid receptors and mK_{ATP} channel opening were involved [95].

Postconditioning with 1.0 MAC isoflurane attenuated myocardial IR injury in an *in vivo* rabbit model to a similar degree as 0.1 mg/kg morphine given upon reperfusion. Neither 0.5 MAC isoflurane nor 0.05 mg/kg morphine alone had a significant effect, but their combination decreased infarct size markedly. The phosphatidylinositol-3-kinase (PI3K) inhibitor wortmannin and naloxone blocked reductions in myocardial infarct size and apoptotic cell death by isoflurane, morphine and their combination, indicating that morphine enhances volatile anesthetic-induced postconditioning by opioid receptor and PI3K activation *in vivo* [96].

Recently, the intravenous anesthetic propofol was shown to have no effect on remifentanyl-induced cardioprotection, but antagonized sevoflurane-induced cardioprotection against IR injury in a working rat heart model [97]. Zaugg and colleagues evaluated the effects of sevoflurane, remifentanyl, and propofol alone and in combination on the recovery of postischemic left ventricular work and drug-induced alterations in Ca^{2+} overload and sarcoplasmic Ca^{2+} leak [97]. Remifentanyl or sevoflurane, but not propofol, improved recovery of left ventricular mechanical function and decreased diastolic Ca^{2+} overload. Peak ischemic $[\text{Ca}^{2+}]_i$ was lower in remifentanyl- compared with sevoflurane-treated hearts. Interestingly, concomitant administration of propofol with sevoflurane completely abolished the sevoflurane-induced improvement in the recovery of left ventricular function during reperfusion and on postischemic diastolic $[\text{Ca}^{2+}]_i$. In contrast, co-administration of propofol with remifentanyl did not alter remifentanyl-induced improvement in the recovery of left ventricular function and diastolic $[\text{Ca}^{2+}]_i$. Administration of N-2-mercaptopropionyl-glycine (MPG), a ROS scavenger similar to propofol, during sevoflurane also blocked anesthetic-enhanced recovery of left ventricular function and postischemic diastolic $[\text{Ca}^{2+}]_i$ during reperfusion. These results support the concept that small amounts of ROS generated during the administration of volatile anesthetics play a pivotal role to trigger volatile anesthetic-induced preconditioning [98, 99]. They also indicate that the mechanism of remifentanyl-induced PC may be different from that of sevoflurane. Thus, interactions among propofol, volatile anesthetics and opioids may influence indices of IR injury and may explain why some clinical trials find no signs of protection [100], whereas other clinical trials and animal studies have shown that volatile anesthetics can produce cardioprotection.

7. Opioid-Induced Cardioprotection in Humans

A large number of animal studies have shown that opioid receptor activation induces cardioprotection, through endogenous release of opioid peptides during IPC or remote PC, or by exogenously administered opioid receptor agonists. There is also a growing body of evidence that this also occurs in humans.

7.1. In Vitro Studies

Transcripts for δ -receptors were found in human atrial trabeculae isolated from patients undergoing coronary artery bypass grafting (CABG). Stimulation of δ -receptors by their agonist DADLE protected trabeculae against simulated hypoxia/reoxygenation injury to a similar degree as IPC. This effect was blocked by a mK_{ATP} channel antagonist [18]. In another *in vitro* study, developed force measured in human atrial trabeculae 60 min after reoxygenation was significantly improved by clinically relevant concentrations of

remifentanyl or sufentanyl when administered before, during and after hypoxia [101]. These findings indicate direct cardioprotection since neither remifentanyl nor sufentanyl produced direct inotropic or lusitropic effects in human atrial myocardium. The absence of inotropic or lusitropic effects of fentanyl in human atrial tissue is less clear, however [102, 103].

Long-term opioid use may reduce the incidence and/or the extent of myocardial infarction by decreasing or even reversing atherosclerosis. A postmortem analysis of the incidence of coronary artery disease (CAD) in 98 methadone or opioid users vs 97 frequency-matched non-users revealed that chronic opioid use may reduce the severity of CAD [104]. Multiple logistic regression analysis yielded an odds ratio of 0.43 (0.20 to 0.94 confidence interval) for opioid use after adjustment for potentially confounding factors.

7.2. In Vivo Studies

Patients subjected to repeated balloon inflations during coronary angioplasty demonstrated significant reductions in pain scores and ST-segment changes during the second balloon inflation compared to the first, a correlate of IPC. However, this effect was not observed in patients who had received the nonspecific opioid receptor antagonist naloxone [105]. These findings suggest mediation of IPC by opioid receptor activation in humans.

In contrast, the PROFIT trial in which 276 elective coronary angioplasty patients were randomized to receive either 5 mg diazepam sublingually or 50 or 100 µg fentanyl IV at least 5 min prior to the first balloon inflation failed to show differences in troponin T elevation or postprocedural myocardial infarction [106]. However, the negative results of the study may be explained by the weaker affinity of fentanyl for the δ -receptor compared to morphine and limited statistical power. The beneficial effects of opioids during cardiac surgery have also been investigated. Global myocardial function as assessed by intraoperative echocardiography following cardiopulmonary bypass in 46 patients was improved after administration of 40 mg morphine compared to 1000 µg fentanyl [107]. Postoperative concentrations of markers of cardiac IR injury such as brain natriuretic peptide or troponin I, however, were not different between the groups. The study was subsequently criticized for a variety of limitations [108]. Interestingly, the same group reported that morphine, but not fentanyl significantly suppressed inflammatory responses as assessed with IL-6, CD 11b, and CD 18 [109]. The authors speculated that greater morphine affinity for the δ -receptor [107] or the μ_3 -receptor [109] and enhanced K_{ATP} channel activation compared to fentanyl may have been responsible for their differential findings. In a small clinical trial, troponin I was markedly reduced up to two days postoperatively following a 5 µg/kg remifentanyl bolus over 10 min before off-pump CABG [110]. Given the small sample size of only twelve patients per group, this positive finding was surprising since both groups received intermittent fentanyl (in addition to a 5–10 µg/kg bolus during induction), as well as various other potentially cardioprotective drugs, e.g. isoflurane and morphine, all of which would be expected to minimize the detection of differences between the groups. Surrogates of cardiac function or long-term outcome were not measured.

Similarly, in a study involving 40 patients undergoing on-pump CABG 1 µg/kg remifentanyl bolus followed by a 0.5 µg/kg/min infusion for 30 min after anesthetic induction, but before sternotomy, resulted in lower troponin I and CK-MB levels for 12 and 24 hrs, respectively.

Both the study and the control group received fentanyl (5 µg/kg followed by 20 µg/kg). In contrast to the study by Xu and colleagues [110], only propofol was given for maintenance of general anesthesia to avoid the cardioprotective effect of a volatile anesthetic [111]. Interestingly, the need for inotropic support after bypass was lower and time to extubation was 2 hrs shorter in the remifentanyl group. There was also a trend for overall decreased length of ICU and hospital stay in the remifentanyl group, but indices of cardiac function and long-term outcome were not assessed. Remifentanyl given throughout the CABG procedure at 0.5 µg/kg/min and discontinued short before the patient's transfer to the ICU was associated with reduced hemodynamic and metabolic responses during surgery, but also caused a marked cardiovascular depression in the early postoperative phase as evidenced by lower cardiac output, left ventricular stroke work index and mixed venous oxygen saturation [112]. No differences in troponin I or CK-MB concentrations were observed between groups. The interpretation of the results of this study is also confounded by its small size (only 10 patients per group), the use of the volatile anesthetic isoflurane, and unmatched confounders (e.g. history of prior MI) between the groups. Nevertheless, the results of these three studies suggest that a short period of high-dose remifentanyl before cardiopulmonary bypass may confer a pharmacological PC effect above and beyond that provided by high-dose fentanyl. Prolonged high-dose infusion of remifentanyl, however, may not only be of no benefit, but could contribute to postoperative hypotension. Remifentanyl PC groups may have been more efficacious than fentanyl for several reasons, one being different opioid receptor affinity (see section 5) and differential cross-talk among opioid receptors (see section 4.4). A more plausible explanation for the observed results, however, is the difference in the opioid dose administered: for example, the unique properties and infusion time-independent context-sensitive half-life of remifentanyl [85, 113], in contrast to fentanyl, likely produced a more rapid onset of high opioid concentrations and, theoretically, activation of more opioid receptors on cardiomyocytes leading to enhanced cardioprotection. Rapid recovery upon termination of its infusion may bridge the at first glance seemingly incompatible needs to employ high opioid concentrations to trigger PC and their avoidance to enable fast recovery.

The cardioprotective effects of opioids may also be modified by other pharmacological actions of specific drugs. For example, oral administration of tramadol the evening before CABG surgery was not cardioprotective compared to remote IPC [114]. Troponin I concentrations were mildly decreased by remote IPC 8 hrs postoperatively compared to control patients; in contrast, patients receiving PO tramadol demonstrated higher troponin I levels for up to 24 hrs after surgery. These results, however, need to be interpreted with caution: tramadol activates a variety of receptors including opioid, noradrenergic and serotonergic receptors; particularly the latter may have been responsible for the worsening, especially since a relatively high dose of tramadol was given (400 mg). A higher proportion of patients with low left ventricular ejection fraction and a lower proportion of patients on HMG coenzyme A reductase inhibitors (statins) were included in the tramadol group. The latter has been shown to reduce cardiac morbidity and mortality independently of antihyperlipidemic effects [115, 116].

Taken together, evidence from both *in vitro* and *in vivo* human studies supports a role for endogenously produced and/or exogenously administered opioids in protecting the human

heart against myocardial IR injury, an encouraging contrast to the challenges of translating laboratory findings on cardioprotection by volatile anesthetics into clinical practice [91, 92].

8. Future Directions

Controversy remains regarding the role for different opioid receptor subtypes to mediate cardioprotection based on pharmacological *vs* molecular characterization. For example, 12 opioid receptor subtypes have been pharmacologically identified, including μ_1 , μ_2 , μ_3 , δ_1 , δ_2 , κ_1 , κ_{1a} , κ_{1b} , κ_2 , κ_{2a} , κ_{2b} , and κ_3 [117]. However, only one gene exists for each μ -, δ -, and κ -opioid receptor, and knockout of a single receptor subtype removes all function associated with that particular receptor [118]. Dietis and colleagues suggest several possibilities to reconcile contradicting findings based on pharmacological *vs* molecular studies. These include a) alternate splicing of a common gene product, b) cross-talk and dimerization among receptors (discussed in section 4.4), c) interaction of common gene products with other receptors/signaling molecules, and d) a combination of all of these factors [117].

Rational drug design to further attenuate myocardial IR injury will require a multidisciplinary approach. Incorporation of gene expression technology and bioinformatic tools is indispensable in structure based design [119]. Further delineation of the mechanisms of activation of opioid receptors and intracellular signaling pathways by endogenous and exogenous ligands to achieve myocardial salvage is essential. Future research should be directed at elucidating the role of splice variants of a common gene product or of receptor dimerization as important modulators of opioid induced protection in myocardium.

9. Summary

Impressive evidence from experiments conducted in various *in-vitro* and *in-vivo* animal and human studies has demonstrated significant opioid-mediated protection against myocardial IR injury. Three different opioid receptor subtypes δ , μ , κ appear to play differential roles in producing this effect. κ - and δ -receptors are expressed in cardiomyocytes, and their activation by endogenously released opioid peptides or exogenously administered opioids appears to be involved in direct myocardial protection. In contrast, the role of μ -receptors to produce myocardial protection is less clear and requires additional investigations. Interestingly, activation of μ -receptors in the CNS, e.g. by IT administration of morphine, appears to exert an indirect cardioprotective effect possibly mediated through activation of calmodulin and the release of calcitonin gene-related peptide. Interpretation of the current literature, however, is complicated because all three opioid receptor subtypes are present in the CNS and could contribute to indirect cardioprotection. In addition, many previous investigations used opioid receptor agonists and antagonists that are not or only partially selective. Clinically used opioids such as morphine, fentanyl and remifentanyl have different affinities to receptor subtypes rendering conclusions regarding subtype involvement in opioid-mediated cardioprotection challenging. All three opioid receptor subtypes demonstrate significant cross-talk among each other and with G-protein coupled receptors. Interactions with other cardioprotective drugs, e.g. volatile anesthetics, also modulate the response to opioid receptor activation during IR injury. Additional factors that modify

opioid-induced cardioprotection include route of administration, dose, and timing in relation to myocardial IR.

In summary, current evidence indicates that opioids produce important effects to protect myocardium against IR injury. Opioid receptor-mediated cardioprotection through ultra-short acting drugs like remifentanyl and remote IPC represent promising approaches to decreasing IR injury, but additional large scale clinical trials to validate early findings are needed.

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Figure 1.

Summary of the important signaling pathways involved in mediating opioid-induced cardioprotection. δ-OR = δ-opioid receptor, κ-OR = κ-opioid receptor, μ-OR = μ-opioid receptor, A₁ = adenosine A₁-receptor, β = adrenergic β-receptor, SarcK_{ATP} = sarcolemmal K_{ATP} channels, mK_{ATP} = mitochondrial K_{ATP} channels, mPTP = mitochondrial permeability transition pore, and ROS = reactive oxygen species.

Table 1

Endogenous and Exogenous Opioids and Opioid Receptors

	Opioids	Receptors
Endogenous	Met-Enkephalin	δ
	Leu-Enkephalin	δ
	β -Endorphin	μ
	Dynorphin	κ
	Nociceptin	OLR1
	Morphine	μ (+++), δ (+), κ (+)
	Fentanyl	μ
Exogenous	Remifentanyl	μ
	Butorphanol	κ (partial agonist, μ antagonist)
	Methadone	μ
	Sufentanyl	μ
	BW373U86	δ
	TAN-67	δ
	Fentanyl isothiocyanate	δ
	SNC-121	δ
	U50,488	κ
	Eribis peptide 94	μ and δ