Available online at www.sciencedirect.com

SCIENCE DIRECT

Vascular Pharmacology 42 (2005) 211–218

Vascular Pharmacology

www.elsevier.com/locate/vph

Review

Opioid-induced preconditioning: Recent advances and future perspectives

Jason N. Peart*, Eric R. Gross, Garrett J. Gross

Department Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Rd, Milwaukee, WI, USA

Abstract

Opioids, named by Acheson [Martin, W.R., 1967. Opioid antagonists.Pharmacol Rev. 19, 463–521] for compounds with morphine-like actions despite chemically distinct structures, have received much research interest, particularly for their central nervous system (CNS) actions involved in pain management, resulting in thousands of scientific papers focusing on their effects on the CNS and other organ systems. A more recent area which may have great clinical importance concerns the role of opioids, either endogenous or exogenous compounds, in limiting the pathogenesis of ischemia–reperfusion injury in heart and brain.

The role of endogenous opioids in hibernation provides tantalizing evidence for the protective potential of opioids against ischemia or hypoxia. Mammalian hibernation, a distinct energy-conserving state, is associated with depletion of energy stores, intracellular acidosis and hypoxia, similar to those which occur during ischemia. However, despite the potentially detrimental cellular state induced with hibernation, the myocardium remains resilient for many months. What accounts for the hypoxia-tolerant state is of great interest. During hibernation, circulating levels of opioid peptides are increased dramatically, and indeed, are considered a "trigger" of hibernation. Furthermore, administration of opioid antagonists can effectively reverse hibernation in mammals. Therefore, it is not surprising that activation of opioid receptors has been demonstrated to preserve cellular status following a hypoxic insult, such as ischemia–reperfusion in many model systems including the intestine [Zhang, Y., Wu, Y.X., Hao, Y.B., Dun, Y., Yang, S.P., 2001. Role of endogenous opioid peptides in protection of ischemic preconditioning in rat small intestine. Life Sci. 68, 1013–1019], skeletal muscle [Addison, P.D., Neligan, P.C., Ashrafpour, H., Khan, A., Zhong, A., Moses, M., Forrest, C.R., Pang, C.Y., 2003. Noninvasive remote ischemic preconditioning for global protection of skeletal muscle against infarction. Am. J. Physiol. Heart Circ. Physiol. 285, H1435–H1443], the CNS [Borlongan, C.V., Wang, Y., Su, T.P., 2005. Delta opioid peptide (d-ala 2, d-leu 5) enkephalin: linking hibernation and neuroprotection. Front Biosci. 9, 3392–3398] and the myocardium [Romano, M.A., Seymour, E.M., Berry, J.A., McNish, R.A., Bolling, S.F., 2004. Relative contribution of endogenous opioids to myocardial ischemic tolerance. J Surg Res. 118, 32–37; Peart, J.N., Gross, G.J., 2004a. Exogenous activation of delta- and kappa-opioid receptors affords cardioprotection in isolated murine heart. Basic Res Cardiol. 99(1), 29–37].

For the purpose of this review, we will focus primarily on the protective effects of opioids against post-reperfusion myocardial stunning and infarction.

Keywords: Opioids; Preconditioning; Ischemia; Reperfusion; G-protein coupled receptors

Contents

* Corresponding author. Tel.: +1 414 456 8872; fax: +1 414 456 6545.
E-mail address: jpeart@mcw.edu (J.N. Peart).

1537-1891/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.vph.2005.02.003

1. Opioid receptor subtypes

The functional effects of opioids are mediated through activation of opioid receptors by their respective peptides. Endogenous opioid peptides, the endorphins, dynorphins and enkephalins associate with the μ-, κ-, and δ-opioid receptors, respectively. The family of opioid receptors, which are classical Gi-protein coupled receptors (GPCR) which inhibit adenylyl cyclase, are separated into three primary sub-groups, the μ-, κ-, and δ-opioid receptors, all of which have been previously cloned. Based upon pharmacological evidence, the κ-, and δ-opioid receptor subfamilies may also include κ_1, κ_2, and δ_1, δ_2 receptors, respectively. For a comprehensive review of opioid receptors and their cardiovascular effects, see the recent review by Pugsley (2002).

Importantly, myocardial cells are sites of opioid peptide synthesis, storage and release (Barron et al., 1995), which are elevated during episodes of stress, such as ischemia (Eliasson et al., 1998). Furthermore, both κ-, and δ-opioid receptors are localized to the myocardium, while, based on binding and gene expression studies, μ-opioid receptors are absent from the adult myocardium. Indeed, mRNA for the enkephalin precursor, preproenkephalin, is highest in the ventricle as opposed to any other organ (Howells et al., 1986). In light of this, it has been suggested that opioids play an intrinsic role in the mediation and regulation of cardiovascular function in both normal and diseased states.

2. Exogenous and endogenous opioids

With the release of opioid peptides during stress, we and others have proposed that opioids may be part of the myocardium's endogenous protective response to ischemia–reperfusion injury. Indeed, Bolling and colleagues conducted a series of studies in which, using cardioplegia and hypothermia to simulate an hibernation-like state, they demonstrated that administration of opioid antagonists, and in particular δ-opioid antagonists, during ischemia did indeed limit functional recovery when compared to untreated hearts (Bolling et al., 2001; Romano et al., 2004). In line with a rapidly growing body of research (reviewed by Schultz and Gross (2001) and Gross (2003), exogenous activation of opioid receptors leads to a preconditioned state.

While there is little doubt regarding the efficacy of opioid receptor activation to produce an ischemia-tolerant state, with activation of either the κ- or the δ-receptor subtypes typically reported as being cardioprotective, there is some controversy regarding the receptor subtype primarily responsible for the beneficial effect of opioids on the heart. Schultz originally reported that both μ- and κ-antagonists failed while δ-antagonists successfully abolished the protective effects of ischemic preconditioning, suggesting little role for the μ- or κ-OR receptor. Furthermore, Aitchison et al. (2000) and Coles et al. (2003) reported detrimental effects of κ-OR activation. Aitchison et al. (2000) reported that κ-OR activation increased infarct size, while Coles et al. (2003) reported that blockade of the κ-OR abrogated reperfusion arrhythmias. In contrast, Wang et al. (2001), demonstrated that κ- but not δ-OR activation mediated both the anti-arrhythmic and infarct limiting effects of IPC.

For a comprehensive review concerning the role of opioid receptors in both acute and delayed preconditioning, see Gross (2003) review.

Recently, much research in the ischemia–reperfusion field has focused on delineating the temporal characteristics of acute pharmacological preconditioning, with evidence shifting the paradigm from ischemic protection to reperfusion-mediated protection. However, the impact of opioids administered at reperfusion has gone relatively unassessed. It should be noted, of course, that it is difficult to separate the effects of opioids on ischemia versus reperfusion injury, and one should be cautious in doing so. Most, if not all, previous studies examining the cytoprotective effects of opioid receptor stimulation employed a protocol consisting of pre-ischemic treatment/activation. Particularly in the case of in vivo models, agonists given prior to ischemia will be present upon reperfusion, unless the half-life is very short, thus making it difficult to separate the two phases. Isolated heart preparations may give some additional benefit due to ligand washout with perfusion. Regardless, only a few studies have examined the effects of opioid agonist administration solely at reperfusion. Recently, Chen et al. (2004) examined the effects of the selective κ-opioid agonist, BRL52537, upon reperfusion in a model of stroke in the rat. These authors reported that BRL52537 treatment robustly reduced infarct size, even when administered up to 4 h following the onset of reperfusion. In a myocardial infarct model of ischemia–reperfusion injury, Gross et al. (2004) reported a significant reduction in infarct development following activation of opioid receptors at reperfusion with either morphine or the selective δ-OR ligand, BW373U86. Gross et al. (2004) also demonstrated that the cardioprotective effects of opioid stimulation at reperfusion are mediated via glycogen synthase kinase beta (GSK-β) and the phosphatidylinositol-3 kinase (PI3K) pathway. Recent, unpublished data accumulated in our laboratory also

indicate that activation of the κ-receptor upon reperfusion, in either the isolated perfused murine or intact rat heart model, provides a robust infarct-sparing effect.

3. Signaling mechanisms

Opioid receptors are G-protein coupled receptors (GPCR) whose activation inhibits adenylyl cyclase, typical of many cardioprotective GPCRs (adenosine, bradykinin, etc.). The post-receptor signaling following opioid receptor activation has not been well defined and is controversial, however, it appears to follow many of the same pathways as the prototypical cardioprotective GPCRs. Recently, Cohen et al. (2001), described a scenario for opioids, bradykinin and acetylcholine whereby receptor activation leads to a "burst" of oxygen-derived free radicals leading to opening of the mitochondrial K_{ATP} channel (mitoK_{ATP}). The opening of the mitoK_{ATP} channel is often considered to be the end-effector in myocardial preconditioning. Interestingly, the protective effect of adenosine does not appear to be mediated via this same pathway, demonstrating that it is not safe to assume that all GPCRs mediate cardioprotection via the same pathway. As such, we will briefly discuss previously reported signaling mechanisms proposed to be responsible for opioid-mediated cardioprotection.

4. Non-receptor mediated mechanisms

As the μ-OR receptor is considered absent from adult myocytes, few studies have assessed the role of the μ-receptor in ischemic tolerance. Regardless, a recent study by Zhang et al. (2004) demonstrated that remifentanil, a short-acting μ-OR agonist, afforded similar protection to IPC. Interestingly, the protective effects of remifentanil could be blocked by agonists selective for μ-, κ- and δ-ORs, while IPC was only sensitive to κ- and δ-OR antagonists, suggesting a peripheral action via the μ-OR component. This peripheral action may involve attenuation of inflammatory products, known to exacerbate ischemia–reperfusion injury in an in vivo model. Previously, Wang et al. (1998) reported that morphine preconditioning limited neutrophil activation via neutrophil endopeptidase. Indeed, pretreatment with the NEP inhibitor, phosphoramidon, attenuated the infarct-sparing effect of morphine-induced preconditioning. In addition, Oh (2002) demonstrated that fentanyl attenuated the increase in the inflammatory cytokines, TNF-α and interleukin-1β, during cerebral ischemia and reperfusion. Taken together, these data provide evidence that μ-OR activation may indeed afford cardioprotection in an in vivo model based upon the attenuation of inflammation.

Opioid agonists, in particular, κ-OR agonists, may also afford cardioprotection via modulation of ion channels. Several lines of evidence support an ion channel blocking property of KOR agonists which appears to be independent of KOR activation. U50,488, along with other KOR agonists, has been shown to prolong the P–R interval and QRS duration, along with inhibiting contractile function in isolated rat hearts (Pugsley et al., 1993) suggesting that these agents may block Na+ and K+ channels. These effects were not blocked by naloxone (Pugsley et al., 1993, 1998). KOR agonists may also antagonize L-type Ca2+ channels (Kasper et al., 1992). Indeed, the apparent receptor-independent antiarrhythmic actions of KOR agonists may be due to direct blockade of cardiac Na+, K+, or Ca2+ channels (Pugsley et al., 1992, 1993, 1998).

However, there is evidence to suggest that the KOR agonists may, indeed, elicit their actions on ion channels via the KOR. Alterations in the $[Ca2+]_i$ transient produced by U50,488 appears to be mediated via a pertussis toxin-sensitive G protein (Bian et al., 1998; Sheng et al., 1997). Analgesia induced by U50,488 appears to be mediated by pertussis toxin-sensitive G proteins coupled to L-type Ca2+ channels (Gullapalli and Ramarao, 2002). Furthermore, the arrhythmogenic action of U50,488 coupled to a rise in $[Na+]_i$ and $[Ca2+]_i$ may be due to a protein kinase C/Na+–H+ exchange pathway (Bian et al., 2000) and involves phosphlipase C and inositol 1,4,5 triphosphate (Bian et al., 1998; Sheng et al., 1997). Indeed, a report by Zhang et al. (1997) suggests that Ca2+ channel blockers compete with KOR agonists in binding to the KOR in a dose-dependent manner.

5. Receptor mediated mechanisms

As mentioned previously, OR activation appears to follow similar post-receptor events as other GPCRs. Much research has demonstrated an imperative role for the mitoK_{ATP} channel in both acute and delayed opioid-mediated cardioprotection in many models (Cohen et al., 2001; Patel et al., 2002a; Peart and Gross, 2003; Seymour et al., 2003). Indeed, the mitoK_{ATP} channel is typically associated with acute and delayed ischemic and pharmacological preconditioning. Interestingly, we have recently shown that the sarcK_{ATP} channel may be the trigger for delayed protection mediated by SNC-121, a selective δ-OR agonist. Administration of SNC-121 leads to a rapid shortening of the action potential. This effect on the action potential, as well as the development of ischemic tolerance 24 h later, was abolished following pretreatment with the sarcK_{ATP} channel blocker, HMR-1098 (Patel et al., 2002b). While the temporal characteristics of OFRs is controversial, that is, do OFRs lead to an opening of the mitoK_{ATP} channel (Zhang et al., 2002), or does opening of the mitoK_{ATP} channel lead to a burst of OFRs responsible for kinase activation and signaling (Forbes et al., 2001; Samavati et al., 2002), the requirement for OFR in opioid preconditioning is well documented. We and numerous others have reported an integral role for OFR release following treatment with opioid agonists. Treatment with a free-radical scavenger,

such as 2-MPG abolished the cardioprotection afforded by both κ-OR (Cao et al., 2004a,b) and δ-OR activation (Cohen et al., 2001; Patel et al., 2001; Peart and Gross, 2003). Interestingly, opioid peptides, particularly the enkephalins, exhibit some free-radical scavenging ability (Coccia et al., 2001). Moreover, Rosenberger et al. (2001), in a model utilizing primary astrocytes, demonstrated that oxidant damage lead to an increase in proenkephalin (pENK) expression. Following H_2O_2 exposure, levels of pENK were elevated, as was phosphorylation of ERK1/2 and p38 MAPK. Curiously, pretreatment with either a MEK (PD98059) or p38 MAPK (SB202190) inhibitor also abolished the oxidant-induced expression of pENK, suggesting a role of endogenous opioids downstream of OFRs.

Similarly, many authors report an integral role for the activation and translocation of PKC, a downstream mediator, following OR-induced preconditioning. However, as with other preconditioning protocols, the isoform involved is still under much debate. Fryer et al. (2001a) has previously demonstrated translocation of PKC isoforms following δ-OR activation. Blockade of the PKC-δ isoform, which was translocated to the mitochondria, abolished the infarct-sparing effects of δ-OR preconditioning without effecting the translocation of the other PKC isoforms. In contrast, a later study by Fryer et al. (2002) failed to link PKC-δ to IPC-mediated protection, perhaps suggesting differences in the signal transduction between IPC and OR activation. Other studies have shown PKC blockade to prevent OR signaling and protection, however, these studies have primarily used non-selective inhibitors (Cao et al., 2004b; Seymour et al., 2003), and as such were unable to delineate between the isoforms. Additionally, Barrere-Lemaire et al. (2005) reported that morphine-induced preconditioning reduced apoptosis induced by simulated ischemia–reperfusion via an IP_3-sensitive signaling pathway.

OR activation is also known to stimulate the phosphatidylinositol-3 kinase (PI3K) pathway (Ai et al., 1999; Cao et al., 2004b). The activation of this signaling pathway is well documented to be an axis of signaling for cardioprotection (Gross et al., 2004). Interestingly, Kam et al. (2004) recently reported that all three receptor subtypes lead to an up-regulation of c-Jun N-terminal kinase (JNK), previously suggested to play a role in preconditioning (Fryer et al., 2001b), however, only μ-OR activation of JNK was PI3K-dependent. Downstream mediators of the PI3K pathway deemed to be essential for opioid preconditioning include GSK-3beta and the mammalian target of rapamycin (mTOR) (Gross et al., 2004) and p38 MAPK (Fryer et al., 2001c). Noteworthy is the study recently documented by Kramer and Simon (2000), who reported that μ- and δ-OR agonists retain the ability to activate MAPK in the absence of receptor internalization. Tong et al. (2004) recently reported a necessity for receptor internalization to mediate post-receptor signaling following ischemic preconditioning.

Activation of extracellular signal regulated kinase (ERK1/2), another member of the MAPK family (p44/42 MAPK), has also been shown to be involved in opioid-mediated cardioprotection, perhaps in parallel to p38 MAPK (Fryer et al., 2001c). Indeed, all three ORs can activate ERK, via transactivation of EGF receptors in transfected HEK cells (Schulz et al., 2004). This is supported by Belcheva et al. (1998), who also suggested that opioid-mediated activation of ERK is reliant upon the betagamma subunits of Gi/o proteins and Ras. Moreover, Cao et al. (2004b) also demonstrated that cardioprotection afforded by δ-OR is mediated via a Src-dependent EGFR transactivation and the PI3K and MAPK pathways.

Delayed cardioprotection afforded by opioids may also incorporate iNOS as an upstream mediator of COX-2 (Kodani et al., 2002; Patel et al., 2004). Supporting this theory, Jiang et al. (2004), has previously shown an absence of morphine-mediated cardioprotection in iNOS gene-knockout mice. Our laboratory reported that iNOS and COX-2 are only required during the mediation phase and not the trigger phase, as iNOS or COX-2 inhibition during δ-OR stimulation fails to alter the infarct-sparing outcome in a delayed preconditioning model.

Heat shock proteins, as with IPC, may be linked to opioid receptor activation. Qi et al. (2004) and Liu et al. (2004), individually indicated that κ-OR stimulation produces an increased expression of HSP70 leading to an ischemia-tolerant state. Alternatively, heat shock protein expression, induced via whole body hyperthermia, leads to a cardioprotective phenotype. This protection is abrogated by pretreatment with the non-selective opioid antagonist, naloxone, without altering HSP70 expression.

6. Opioid receptor cross-talk

Recently we demonstrated a cross-talk relationship between opioid and adenosine receptors (Peart and Gross, 2003). We reported that the infarct-sparing effect afforded by morphine could be blocked by the adenosine A1 receptor (A1AR) antagonist, DPCPX, while the protective effect of the A1AR agonist, CCPA, was abolished by the δ-OR antagonist, BNTX. Moreover, BNTX failed to antagonize the bradycardic effects of CCPA, suggesting that direct A_1AR receptor activation was most likely not being inhibited by BNTX. While A_1AR antagonism has previously been shown to abolish opioid-mediated protection in the rat heart (Kato et al., 2000), these data are the first to suggest that DOR blockade inhibits A_1AR-mediated protection. Furthermore, an interaction between opioids and adenosine has been previously documented. Binding of [^{3}H]DPCPX is reduced in the brain of μ-opioid receptor knockout mice (Bailey et al., 2002). DORs are also downregulated in μ-knockout mice (Kitchen et al., 1997). Concentrations of cortical A_1AR binding sites are increased following 72 h of morphine treatment in mice (Kaplan et al.,

1994) and morphine produced a concentration-dependent release of adenosine (Sandner-Kiesling et al., 2001). Moreover, bi-directional cross-withdrawal syndromes were evident when adenosine agonist pretreated rats were administered the opioid antagonist, naloxone, and when rats pretreated with morphine were given DPCPX or DMPX (Coupar and Tran, 2001), two adenosine receptor antagonists. While these studies demonstrate a tight coupling between opioids and adenosine in the CNS, the present data suggest a tangible link in cardiac tissue as well.

In addition, cross-talk between opioids and other receptors/transduction systems has also been documented. The effect of interleukin-2 (IL-2) on the $[Ca2+]_i$ transient and contraction appears to be mediated via a κ-OR via a pertussis toxin sensitive GPCR and phospholipase C dependent mechanism (Cao et al., 2002). Furthermore, the cardioprotective effects of IL-2 following ischemia–reperfusion in the isolated rat heart, were effectively blocked by a κ-OR antagonist, while a selective δ-OR antagonist had no effect upon the IL-2-mediated cardioprotection (Cao et al., 2004a).

There are also numerous reports outlining cross-talk between opioids and the beta-adrenergic system in the heart (for review, see Pepe et al., 2004). This is an interesting interaction since ORs are Gi GPCR and the beta-adrnerergic receptor is a classical Gs GPCR, stimulating adenylyl cyclase. In brief, opioid peptides and catecholamines are coreleased from neuronal terminals following sympathetic stimulation. It appears that the opioid peptides essentially act as an antagonist to the beta-adrenergic system by limiting the beta-adrenergic increase in cAMP. Furthermore, the cross-talk may also alter signaling characteristics. Curiously, the interaction between opioids and the beta-adrenergic system may be both functional and physical, that is, the interaction may be due to an opposing convergence on downstream signaling, or may be in part due to receptor dimerization.

Recent studies have provided evidence that functional GPCRs heterodimerize, resulting in unique sites with novel ligand-binding properties (George et al., 2000; Gomes et al., 2000; Jordan and Devi, 1999). In addition to alterations in ligand-binding, dimerization may also modify signaling and trafficking properties of these receptors (Pfeiffer et al., 2002). Indeed, receptor heterodimerization may account for receptor subtypes, yet to be cloned, which have only been characterized by pharmacological studies and may also represent receptors for orphan peptides. Moreover, the μ–δ-complexed opioid receptors appear to be sensitive to ligands selective for the δ_2-opoioid receptor subtype (Gomes et al., 2000; Xu et al., 1993). While it has been demonstrated that the μ- and δ- and the κ- and δ-opioid receptors form hetero-oligomers (Gomes et al., 2000; Jordan and Devi, 1999), opioid receptors have been shown to dimerize with other GPCRs, namely β_2-adrenergic and somatostatin receptors (Jordan et al., 2001; Pfeiffer et al., 2002). The μ-OR may also dimerize with alpha2A-adrenergic receptors leading to a functional influence (Jordan et al., 2003), however the functional aspect of this physical interaction has previously been disputed (Zhang and Limbird, 2004). Furthermore, A_1AR can form heterodimers with $P2Y_1$ receptors (Yoshioka et al., 2002) and dopamine D_1 receptors (Gines et al., 2000), while the A_2AR has been shown to exist as an oligomer with dopamine D_2 receptors in living cells (Kamiya et al., 2003). A_1AR and A_2AR antagonists also abolish synergistic actions between opioids and dopamine D_2 receptors (Yao et al., 2003).

It is unclear whether cross-talk and receptor dimerization are always one in the same. While it may be difficult to explain receptor cross-talk in the absence of heterodimerization, the opioid (homo)dimerization results in altered pharmacological characteristics of opioid receptors. That is, the ligand binding characteristics are altered. Regardless, these data would suggest that opioids are a promiscuous receptor family.

7. Chronic opioid preconditioning

We have recently reported that chronic opioid exposure leads to a phenotype consisting of a profound ischemic-tolerant state (Peart and Gross, 2004b). In brief, mice were implanted with slow-release morphine pellets for up to 5 days. The preconditioning effect manifest as early as 3 days post-implantation, while analgesic tolerance was not evident until 4 days. While the cardioprotective phenotype preceded the analgesia tolerance by at least 24 h, we cannot draw a conclusion regarding the pathways involved in both phenomena. Moreover, this protective phenotype persists for at least 48 h following complete opiate withdrawal. Furthermore, this chronic opioid preconditioning protocol is equally effective in aged mice where acute opioid cardioprotection is lost (Peart and Gross, 2004c). Of great interest is that our preliminary studies suggest that this robust protection appears impervious to a wide variety of Gi-receptor and kinase antagonists when administered prior to the index ischemia and subsequent reperfusion.

Kuzume et al. (2003) reported that a 24hr administration of the δ-OR agonist, Met^5-enkephalin (ME) lead to a significant reduction in infarct size in open-chest rabbits. This protection was effectively abrogated by both sarcolemmal and mitoK_{ATP} channel blockade. Curiously, when ME was infused as a chronic 14 day treatment in mice (Kuzume et al., 2004), ischemic tolerance was lost. It is difficult to compare the results of our chronic study with those of Kuzume due to the major differences in the exposure protocol (5 days, as opposed to either 24 h or 14 days).

However, information gleaned from research in the CNS may provide some insight into the potential mechanisms behind chronic opioid preconditioning.

Chronic opioid exposure in the CNS has garnered much research in the past and the knowledge afforded by the CNS

studies may provide an insight into the mechanism of protection seen in the heart. Chronic opioid treatment leads to an increase in the release of endogenous substances such as substance P, calcitonin gene-related peptide (Trang et al., 2002) and adenosine (Halimi et al., 2000). Indeed, chronic morphine exposure increases adenosine receptor sensitivity (Brundege and Williams, 2002). Following chronic activation of opioid receptors, the opioid receptors may convert from inhibitory (Gi-coupled) to excitatory (Gs-coupled) mode (Crain and Shen, 1992). Furthermore, chronic exposure to opioids produce a superactivation of adenylyl cyclase (Varga et al., 2003). Moreover, chronic exposure may afford activation of multiple redundant signaling pathways (Varga et al., 2003).

Revealing the mechanism by which chronic opioid exposure affords such a dramatic cardioprotective phenotype may have powerful ramifications regarding protection of the myocardium following cardiac surgical interventions.

8. Summary

While the exact mechanisms may not be fully understood, there is a wealth of information supporting the hypothesis that opioids can confer an acute and chronic cardioprotective state. While μ-OR activation may lead to protection via a reduction of the inflammatory response including neutrophil activation, κ- and δ-OR activation leads to a complex downstream signaling complex to induce preconditioning. The acute protection provided by opioids appears to be tightly linked to adenosine receptors, however, the exact mechanism is still unclear, and the recent documentation of chronic opioid-induced preconditioning may provide important new insights to greatly improve and extend the window of pharmacological cardioprotection.

The current use of opioids post-operatively and as an approved treatment for both acute and chronic pain would potentially negate the need for a long period of drug development before opioids coould be approved for use as cardioprotective agents, making the future of opioids appealing. In particular, development of a peripherally active opioid that lacks CNS effects would be particularly interesting.

References

Addison, P.D., Neligan, P.C., Ashrafpour, H., Khan, A., Zhong, A., Moses, M., Forrest, C.R., Pang, C.Y., 2003. Noninvasive remote ischemic preconditioning for global protection of skeletal muscle against infarction. Am. J. Physiol., Heart Circ. Physiol. 285, H1435–H1443.

Ai, W., Gong, J., Yu, L., 1999. MAP kinase activation by mu opioid receptor involves phosphatidylinositol 3-kinase but not the cAMP/PKA pathway. FEBS Lett. 456, 196–200.

Aitchison, K.A., Baxter, G.F., Awan, M.M., Smith, R.M., Yellon, D.M., Opie, L.H., 2000. Opposing effects on infarction of delta and kappa opioid receptor activation in the isolated rat heart: implications for ischemic preconditioning. Basic Res. Cardiol. 95, 1–10.

Bailey, A., Matthes, H., Kieffer, B., Slowe, S., Hourani, S.M., Kitchen, I., 2002. Quantitative autoradiography of adenosine receptors and NBTI-sensitive adenosine transporters in the brains and spinal cords of mice deficient in the mu-opioid receptor gene. Brain Res. 943, 68–79.

Barrere-Lemaire, S., Combes, N., Sportouch-Dukhan, C., Richard, S., Nargeot, J., Piot, C., 2005. Morphine mimics the antiapoptotic effect of preconditioning via an Ins(1,4,5)P3 signaling pathway in rat ventricular myocytes. Am. J. Physiol., Heart Circ. Physiol. 288, H83–H88.

Barron, B.A., Jones, C.E., Caffrey, J.L., 1995. Pericardial repair depresses canine cardiac catecholamines and met-enkephalin. Regul. Pept. 59, 313–320.

Belcheva, M.M., Vogel, Z., Ignatova, E., Avidor-Reiss, T., Zippel, R., Levy, R., Young, E.C., Barg, J., Coscia, C.J., 1998. Opioid modulation of extracellular signal-regulated protein kinase activity is ras-dependent and involves Gbetagamma subunits. J. Neurochem. 70, 635–645.

Bian, J.S., Pei, J.M., Cheung, C.S., Zhang, W.M., Wong, T.M., 1998. Phospholipase C inhibitors attenuate arrhythmias induced by kappa-receptor stimulation in the isolated rat heart. J. Mol. Cell. Cardiol. 30, 2103–2110.

Bian, J.S., Pei, J.M., Cheung, C.S., Zhang, W.M., Wong, T.M., 2000. Kappa-opioid receptor stimulation induces arrhythmia in the isolated rat heart via the protein kinase C/Na(+)–H(+)exchange pathway. J. Mol. Cell. Cardiol. 32, 1415–1427.

Bolling, S.F., Badhwar, V., Schwartz, C.F., Oeltgen, P.R., Kilgore, K., Su, T.P., 2001. Opioids confer myocardial tolerance to ischemia: interaction of delta opioid agonists and antagonists. J. Thorac. Cardiovasc. Surg. 122, 476–481.

Borlongan, C.V., Wang, Y., Su, T.P., 2005. Delta opioid peptide (D-ala 2, D-leu 5) enkephalin: linking hibernation and neuroprotection. Front. Biosci. 9, 3392–3398.

Brundege, J.M., Williams, J.T., 2002. Increase in adenosine sensitivity in the nucleus accumbens following chronic morphine treatment. J. Neurophysiol. 87, 1369–1375.

Cao, C.M., Xia, Q., Chen, Y.Y., Zhang, X., Shen, Y.L., 2002. Opioid receptor-mediated effects of interleukin-2 on the [Ca2+]i transient and contraction in isolated ventricular myocytes of the rat. Pflugers Arch. 443, 635–642.

Cao, C.M., Xia, Q., Tu, J., Chen, M., Wu, S., Wong, T.M., 2004. Cardioprotection of interleukin-2 is mediated via kappa-opioid receptors. J. Pharmacol. Exp. Ther. 309, 560–567.

Cao, Z., Liu, L., Van Winkle, D.M., 2004. Met5-enkephalin-induced cardioprotection occurs via transactivation of EGFR and activation of PI3K. Am. J. Physiol., Heart Circ. Physiol.

Chen, T.Y., Goyagi, T., Toung, T.J., Kirsch, J.R., Hurn, P.D., Koehler, R.C., Bhardwaj, A., 2004. Prolonged opportunity for ischemic neuroprotection with selective kappa-opioid receptor agonist in rats. Stroke 35, 1180–1185.

Coccia, R., Foppoli, C., Blarzino, C., De Marco, C., Rosei, M.A., 2001. Interaction of enkephalin derivatives with reactive oxygen species. Biochim. Biophys. Acta 1525, 43–49.

Cohen, M.V., Yang, X.M., Liu, G.S., Heusch, G., Downey, J.M., 2001. Acetylcholine, bradykinin, opioids, and phenylephrine, but not adenosine, trigger preconditioning by generating free radicals and opening mitochondrial K(ATP) channels. Circ. Res. 89, 273–278.

Coles Jr., J.A., Sigg, D.C., Iaizzo, P.A., 2003. Role of kappa-opioid receptor activation in pharmacological preconditioning of swine. Am. J. Physiol., Heart Circ. Physiol. 84, H2091–H2099.

Coupar, I.M., Tran, B.L., 2001. Withdrawal and bidirectional cross-withdrawal responses in rats treated with adenosine agonists and morphine. Life Sci. 69, 779–790.

Crain, S.M., Shen, K.F., 1992. After chronic opioid exposure sensory neurons become supersensitive to the excitatory effects of opioid agonists and antagonists as occurs after acute elevation of GM1 ganglioside. Brain Res. 575, 13–24.

Eliasson, T., Mannheimer, C., Waagstein, F., Andersson, B., Bergh, C.H., Augustinsson, L.E., Hedner, T., Larson, G., 1998. Myocardial turnover of endogenous opioids and calcitonin-gene-related peptide in the human

heart and the effects of spinal cord stimulation on pacing-induced angina pectoris. Cardiology 89, 170–177.
Forbes, R.A., Steenbergen, C., Murphy, E., 2001. Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. Circ. Res. 88, 802–809.
Fryer, R.M., Wang, Y., Hsu, A.K., Gross, G.J., 2001a. Essential activation of PKC-delta in opioid-initiated cardioprotection. Am. J. Physiol., Heart Circ. Physiol. 280, H1346–H1353.
Fryer, R.M., Patel, H.H., Hsu, A.K., Gross, G.J., 2001b. Stress-activated protein kinase phosphorylation during cardioprotection in the ischemic myocardium. Am. J. Physiol., Heart Circ. Physiol. 281, H1184–H1192.
Fryer, R.M., Hsu, A.K., Gross, G.J., 2001. ERK and p38 MAP kinase activation are components of opioid-induced delayed cardioprotection. Basic Res. Cardiol. 96, 136–142.
Fryer, R.M., Hsu, A.K., Wang, Y., Henry, M., Eells, J., Gross, G.J., 2002. PKC-delta inhibition does not block preconditioning-induced preservation in mitochondrial ATP synthesis and infarct size reduction in rats. Basic Res. Cardiol. 97, 47–54.
George, S.R., Fan, T., Xie, Z., Tse, R., Tam, V., Varghese, G., O'Dowd, B.F., 2000. Oligomerization of mu- and delta-opioid receptors. Generation of novel functional properties. J. Biol. Chem. 275, 26128–26135.
Gines, S., Hillion, J., Torvinen, M., Le Crom, S., Casado, V., Canela, E.I., Rondin, S., Lew, J.Y., Watson, S., Zoli, M., Agnati, L.F., Verniera, P., Lluis, C., Ferre, S., Fuxe, K., Franco, R., 2000. Dopamine D1 and adenosine A1 receptors form functionally interacting heteromeric complexes. Proc. Natl. Acad. Sci. U. S. A. 97, 8606–8611.
Gomes, I., Jordan, B.A., Gupta, A., Trapaidze, N., Nagy, V., Devi, L.A., 2000. Heterodimerization of mu and delta opioid receptors: a role in opiate synergy. J. Neurosci. 20, RC110.
Gross, G.J., 2003. Role of opioids in acute and delayed preconditioning. J. Mol. Cell. Cardiol. 35, 709–718.
Gross, E.R., Hsu, A.K., Gross, G.J., 2004. Opioid-induced cardioprotection occurs via glycogen synthase kinase beta inhibition during reperfusion in intact rat hearts. Circ. Res. 94, 960–966.
Gullapalli, S., Ramarao, P., 2002. Role of L-type Ca(2+) channels in pertussis toxin induced antagonism of U50,488H analgesia and hypothermia. Brain Res. 946, 191–197.
Halimi, G., Devaux, C., Clot-Faybesse, O., Sampol, J., Legof, L., Rochat, H., Guieu, R., 2000. Modulation of adenosine concentration by opioid receptor agonists in rat striatum. Eur. J. Pharmacol. 398, 217–224.
Howells, R.D., Kilpatrick, D.L., Bailey, L.C., Noe, M., Udenfriend, S., 1986. Proenkephalin mRNA in rat heart. Proc. Natl. Acad. Sci. U. S. A. 83, 1960–1963.
Jiang, X., Shi, E., Nakajima, Y., Sato, S., 2004. Inducible nitric oxide synthase mediates delayed cardioprotection induced by morphine in vivo: evidence from pharmacologic inhibition and gene-knockout mice. Anesthesiology 101, 82–88.
Jordan, B.A., Devi, L.A., 1999. G-protein-coupled receptor heterodimerization modulates receptor function. Nature 399, 697–700.
Jordan, B.A., Trapaidze, N., Gomes, I., Nivarthi, R., Devi, L.A., 2001. Oligomerization of opioid receptors with beta 2-adrenergic receptors: a role in trafficking and mitogen-activated protein kinase activation. Proc. Natl. Acad. Sci. U. S. A. 98, 343–348.
Jordan, B.A., Gomes, I., Rios, C., Filipovska, J., Devi, L.A., 2003. Functional interactions between mu opioid and alpha 2A-adrenergic receptors. Mol. Pharmacol. 64, 1317–1324.
Kam, A.Y., Chan, A.S., Wong, Y.H., 2004. Phosphatidylinositol-3 kinase is distinctively required for mu-, but not kappa-opioid receptor-induced activation of c-Jun N-terminal kinase. J. Neurochem. 89, 391–402.
Kamiya, T., Saitoh, O., Yoshioka, K., Nakata, H., 2003. Oligomerization of adenosine A2A and dopamine D2 receptors in living cells. Biochem. Biophys. Res. Commun. 306, 544–549.
Kaplan, G.B., Leite-Morris, K.A., Sears, M.T., 1994. Alterations of adenosine A1 receptors in morphine dependence. Brain Res. 657, 347–350.
Kasper, E., Ventura, C., Ziman, B.D., Lakatta, E.G., Weisman, H., Capogrossi, M.C., 1992. Effect of U-50,488H on the contractile response of cardiomyopathic hamster ventricular myocytes. Life Sci. 50, 2029–2035.
Kato, R., Ross, S., Foex, P., 2000. Fentanyl protects the heart against ischaemic injury via opioid receptors, adenosine A_1 receptors and K_{ATP} channel linked mechanisms in rats. Br. J. Anaesth. 84, 204–214.
Kitchen, I., Slowe, S.J., Matthes, H.W., Kieffer, B., 1997. Quantitative autoradiographic mapping of μ-, δ- and κ-opioid receptors in knockout mice lacking the μ-opioid receptor gene. Brain Res. 778, 73–88.
Kodani, E., Xuan, Y.T., Shinmura, K., Takano, H., Tang, X.L., Bolli, R., 2002. Delta-opioid receptor-induced late preconditioning is mediated by cyclooxygenase-2 in conscious rabbits. Am. J. Physiol., Heart Circ. Physiol. 283, H1943–H1957.
Kramer, H.K., Simon, E.J., 2000. Mu and delta-opioid receptor agonists induce mitogen-activated protein kinase (MAPK) activation in the absence of receptor internalization. Neuropharmacology 39, 1707–1719.
Kuzume, K., Wolff, R.A., Amakawa, K., Kuzume, K., Van Winkle, D.M., 2003. Sustained exogenous administration of Met5-enkephalin protects against infarction in vivo. Am. J. Physiol., Heart Circ. Physiol. 285, H2463–H2470.
Kuzume, K., Kuzume, K., Cao, Z., Liu, L., Van Winkle, D.M., 2004. Long term infusion of Met5-enkephalin fails to protect murine hearts against ischemia–reperfusion injury. Am. J. Physiol., Heart Circ. Physiol.
Liu, J., Kam, K.W., Zhou, J.J., Yan, W.Y., Chen, M., Wu, S., Wong, T.M., 2004. Effects of heat shock protein 70 activation by metabolic inhibition preconditioning or {kappa}-opioid receptor stimulation on Ca2+ homeostasis in rat ventricular myocytes subjected to ischemic insults. J. Pharmacol. Exp. Ther. 310, 606–613.
Martin, W.R., 1967. Opioid antagonists. Pharmacol. Rev. 19, 463–521.
Oh, W.S., 2002. Effect of fentanyl on TNF-alpha and IL-1beta levels during global ischemia/reperfusion in rats. Int. J. Tissue React. 24, 11–21.
Patel, H.H., Hsu, A., Moore, J., Gross, G.J., 2001. BW373U86, a delta opioid agonist, partially mediates delayed cardioprotection via a free radical mechanism that is independent of opioid receptor stimulation. J. Mol. Cell. Cardiol. 33, 1455–1465.
Patel, H.H., Ludwig, L.M., Fryer, R.M., Hsu, A.K., Warltier, D.C., Gross, G.J., 2002a. Delta opioid agonists and volatile anesthetics facilitate cardioprotection via potentiation of K(ATP) channel opening. FASEB J. 16, 1468–1470.
Patel, H.H., Hsu, A.K., Peart, J.N., Gross, G.J., 2002b. Sarcolemmal K(ATP) channel triggers opioid-induced delayed cardioprotection in the rat. Circ. Res. 91, 186–188.
Patel, H.H., Hsu, A.K., Gross, G.J., 2004. COX-2 and iNOS in opioid-induced delayed cardioprotection in the intact rat. Life Sci. 75, 129–140.
Peart, J.N., Gross, G.J., 2003. Adenosine and opioid receptor-mediated cardioprotection in the rat: evidence for cross-talk between receptors. Am. J. Physiol., Heart Circ. Physiol. 285, H81–H89.
Peart, J.N., Gross, G.J., 2004a. Exogenous activation of delta- and kappa-opioid receptors affords cardioprotection in isolated murine heart. Basic Res. Cardiol. 99 (1), 29–37.
Peart, J.N., Gross, G.J., 2004b. Morphine-tolerant mice exhibit a profound and persistent cardioprotective phenotype. Circulation 109, 1219–1222.
Peart, J.N., Gross, G.J., 2004c. Chronic exposure to morphine produces a marked cardioprotective phenotype in aged mouse hearts. Exp. Gerontol. 39 (7), 1021–1026.
Pepe, S., van den Brink, O.W., Lakatta, E.G., Xiao, R.P., 2004. Cross-talk of opioid peptide receptor and beta-adrenergic receptor signalling in the heart. Cardiovasc. Res. 63, 414–422.
Pfeiffer, M., Koch, T., Schroder, H., Laugsch, M., Hollt, V., Schulz, S., 2002. Heterodimerization of somatostatin and opioid receptors cross-

modulates phosphorylation, internalization, and desensitization. J. Biol. Chem. 277, 19762–19772.
Pugsley, M.K., 2002. The diverse molecular mechanisms responsible for the actions of opioids on the cardiovascular system. Pharmacol. Ther. 93, 51–75.
Pugsley, M.K., Penz, W.P., Walker, M.J., Wong, T.M., 1992. Cardiovascular actions of the kappa-agonist, U-50,488H, in the absence and presence of opioid receptor blockade. Br. J. Pharmacol. 105, 521–526.
Pugsley, M.K., Saint, D.A., Penz, M.P., Walker, M.J., 1993. Electrophysiological and antiarrhythmic actions of the kappa agonist PD 129290, and its *R*,*R* (+)-enantiomer, PD 129289. Br. J. Pharmacol. 110, 1579–1585.
Pugsley, M.K., Saint, D.A., Hayes, E.S., Kramer, D., Walker, M.J., 1998. Sodium channel-blocking properties of spiradoline, a kappa receptor agonist, are responsible for its antiarrhythmic action in the rat. J. Cardiovasc. Pharmacol. 32, 863–874.
Qi, J.S., Kam, K.W., Chen, M., Wu, S., Wong, T.M., 2004. Failure to confer cardioprotection and to increase the expression of heat-shock protein 70 by preconditioning with a kappa-opioid receptor agonist during ischaemia and reperfusion in streptozotocin-induced diabetic rats. Diabetologia 47, 214–220.
Romano, M.A., Seymour, E.M., Berry, J.A., McNish, R.A., Bolling, S.F., 2004. Relative contribution of endogenous opioids to myocardial ischemic tolerance. J. Surg. Res. 118, 32–37.
Rosenberger, J., Petrovics, G., Buzas, B., 2001. Oxidative stress induces proorphanin FQ and proenkephalin gene expression in astrocytes through p38- and ERK-MAP kinases and NF-kappaB. J. Neurochem. 79, 35–44.
Samavati, L., Monick, M.M., Sanlioglu, S., Buettner, G.R., Oberley, L.W., Hunninghake, G.W., 2002. Mitochondrial KATP channel openers activate the ERK kinase by an oxidant-dependent mechanism. Am. J. Physiol., Cell Physiol. 283, C273–C281.
Sandner-Kiesling, A., Li, X., Eisenach, J.C., 2001. Morphine-induced spinal release of adenosine is reduced in neuropathic rats. Anesthesiology 95, 1455–1459.
Schultz, J.E., Gross, G.J., 2001. Opioids and cardioprotection. Pharmacol. Ther. 89, 123–137.
Schulz, R., Eisinger, D.A., Wehmeyer, A., 2004. Opioid control of MAP kinase cascade. Eur. J. Pharmacol. 500, 487–497.
Seymour, E.M., Wu, S.Y., Kovach, M.A., Romano, M.A., Traynor, J.R., Claycomb, W.C., Bolling, S.F., 2003. HL-1 myocytes exhibit PKC and K(ATP) channel-dependent delta opioid preconditioning. J. Surg. Res. 114, 187–194.
Sheng, J.Z., Wong, N.S., Wang, H.X., Wong, T.M., 1997. Pertussis toxin, but not tyrosine kinase inhibitors, abolishes effects of U-50488H on [Ca2+]i in myocytes. Am. J. Physiol. 272, C560–C564.
Tong, H., Rockman, H.A., Koch, W.J., Steenbergen, C., Murphy, E., 2004. G protein-coupled receptor internalization signaling is required for cardioprotection in ischemic preconditioning. Circ. Res. 94, 1133–1141.
Trang, T., Sutak, M., Quirion, R., Jhamandas, K., 2002. The role of spinal neuropeptides and prostaglandins in opioid physical dependence. Br. J. Pharmacol. 136, 37–48.
Varga, E.V., Yamamura, H.I., Rubenzik, M.K., Stropova, D., Navratilova, E., Roeske, W.R., 2003. Molecular mechanisms of excitatory signaling upon chronic opioid agonist treatment. Life Sci. 74, 299–311.
Wang, T.L., Chang, H., Hung, C.R., Tseng, Y.Z., 1998. Morphine preconditioning attenuates neutrophil activation in rat models of myocardial infarction. Cardiovasc. Res. 40, 557–563.
Wang, G.Y., Wu, S., Pei, J.M., Yu, X.C., Wong, T.M., 2001. Kappa- but not delta-opioid receptors mediate effects of ischemic preconditioning on both infarct and arrhythmia in rats. Am. J. Physiol., Heart Circ. Physiol. 280, H384–H391.
Xu, H., Partilla, J.S., de Costa, B.R., Rice, K.C., Rothman, R.B., 1993. Differential binding of opioid peptides and other drugs to two subtypes of opioid delta ncx binding sites in mouse brain: further evidence for delta receptor heterogeneity. Peptides 14, 893–907.
Yao, L., Fan, P., Jiang, Z., Mailliard, W.S., Gordon, A.S., Diamond, I., 2003. Addicting drugs utilize a synergistic molecular mechanism in common requiring adenosine and Gi-beta gamma dimers. Proc. Natl. Acad. Sci. U. S. A. 100, 14379–14384.
Yoshioka, K., Hosoda, R., Kuroda, Y., Nakata, H., 2002. Hetero-oligomerization of adenosine A1 receptors with P2Y1 receptors in rat brains. FEBS Lett. 531, 299–303.
Zhang, Y.Q., Limbird, L.E., 2004. Hetero-oligomers of alpha2A-adrenergic and mu-opioid receptors do not lead to transactivation of G-proteins or altered endocytosis profiles. Biochem. Soc. Trans. 32 (Pt 5), 856–860.
Zhang, W.M., Wang, H.X., Xia, Q., Wong, T.M., 1997. Inhibition of [3H]-U69593 binding and the cardiac effects of U50,488H by calcium channel blockers in the rat heart. Br. J. Pharmacol. 120, 827–832.
Zhang, Y., Wu, Y.X., Hao, Y.B., Dun, Y., Yang, S.P., 2001. Role of endogenous opioid peptides in protection of ischemic preconditioning in rat small intestine. Life Sci. 68, 1013–1019.
Zhang, H.Y., McPherson, B.C., Liu, H., Baman, T.S., Rock, P., Yao, Z., 2002. H_2O_2 opens mitochondrial KATP channels and inhibits GABA receptors via protein kinase C- in cardiomyocytes. Am. J. Physiol., Heart Circ. Physiol. 282, H1395–H1403.
Zhang, Y., Irwin, M.G., Wong, T.M., 2004. Remifentanil preconditioning protects against ischemic injury in the intact rat heart. Anesthesiology 101, 918–923.