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Morphine stimulates nitric oxide release in human mitochondria

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Abstract The expression of morphine by plants, invertebrate, and vertebrate cells and organ systems, strongly indicates a high level of evolutionary conservation of morphine and related morphinan alkaloids as required for life. The prototype catecholamine, dopamine, serves as an essential chemical intermediate in morphine biosynthesis, both in plants and animals. We surmise that, before the emergence of specialized plant and animal cells/organ systems, primordial multipotential cell types required selective mechanisms to limit their responsiveness to environmental cues. Accordingly, cellular systems that emerged with the potential for recruitment of the free radical gas nitric oxide (NO) as a multi-faceted autocrine/paracrine signaling molecule, were provided with extremely positive evolutionary advantages. Endogenous morphinergic signaling, in concert with NO-coupled signaling systems, has evolved as an autocrine/paracrine regulator of metabolic homeostasis, energy metabolism, mitochondrial respiration and energy production. Basic physiological processes involving morphinergic/NO-coupled regulation of mitochondrial function, with special emphasis on the cardiovascular system, are critical to all organismic survival. Key to this concept may be the phenomenon of mitochondrial enslavement in eukaryotic evolution via endogenous morphine.

Keywords Endogenous morphine · Nitric oxide · Nitric oxide synthase · Mitochondria · Opiate receptor sub-types

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Introduction

It is widely known that the opium poppy (*Papaver somniferum*) synthesizes morphine as a major phytoalexin, and dopamine (DA) serves as an essential precursor in this biosynthetic pathway (Liscombe and Facchini 2008; Liscombe et al. 2005). Molecular phylogenetic analysis indicates that the enzyme (S)-norcoclaurine synthase and benzylisoquinoline (BIQ) alkaloid biosynthetic pathways evolved prior to the emergence of the eudicots (Liscombe and Facchini 2008; Liscombe et al. 2005). Evolutionary pressure has apparently preserved the ability to synthesize chemically authentic morphine, albeit in homeopathic concentrations, throughout animal phyla (Boettcher et al. 2005; Fricchione et al. 2008; Kream et al. 2009; Kream et al. 2007; Kream and Stefano 2006; Stefano and Kream 2010; Zhu et al. 2005a). De novo biosynthesis of morphine was also demonstrated from other small aromatic molecules derived from L-tyrosine (L-TYR) in invertebrate ganglia, mammalian immune cells, and in human cancer cell lines (Kream and Stefano 2006; Zhu et al. 2005a; Zhu et al. 2005b). Accordingly, we have formulated a hypothesis stating that dopamine-expressing signaling systems emerged from the morphine biosynthetic pathway via evolutionary adaptation of key enzymes involved in the modification of L-TYR, DA, 3,4-dihydroxy-L-tyrosine (DOPA), and tyramine (Kream et al. 2009; Kream and Stefano 2006; Stefano and Kream 2007; Stefano and Kream 2010). Operationally, dopamine and endogenous morphinergic signaling systems share a common set of biosynthetic and metabolic enzymes, indicating significant evolutionary retrofitting of primordial enzyme species (Kream et al. 2009; Stefano and Kream 2010).

Endogenous morphine appears to exert a state-dependent down-regulation of cellular excitability, via highly specific receptor-mediated processes, within a highly confined local

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circuitry (Stefano 1998; Stefano et al. 2000a; Stefano et al. 2000b; Stefano and Scharrer 1994). In a previous review, we have suggested that evolutionary pressure drove an adaptive expansion of catecholamine signaling systems in animal phyla commensurate with the acquisition of complex motor activities associated with complex feeding, sexual and protective processes (Stefano and Kream 2007).

Our group and others (Andersen et al. 2013) have provided ample biochemical, pharmacological, and molecular evidence to support the existence and biological importance of two unique 6 transmembrane helical domain (TMH6) opiate receptors, which are expressed from the mu 1 opioid receptor (OPRM1) gene, that are functionally coupled to Ca^{2+}-dependent production and release of NO from the constitutive isozyme of NO synthase (cNOS) (Cadet et al. 2003; Stefano 1998; Stefano et al. 1993; Stefano et al. 2000a; Stefano et al. 2000b; Stefano et al. 1995a; Stefano et al. 1996). We now provide pharmacological evidence that the mitochondrial mu receptor is indeed one of the novel TMH6 opiate receptors. Thus, these TMH6 opiate receptor subtypes are selectively tailored to mediate the cellular regulatory effects of endogenous morphine and related BIQ alkaloids within a highly restricted signaling pathway, involving stimulation of constitutive NO that occurs, in part, within the mitochondria.

Material and methods

HTB-11 cells (ATCC, Manassas, VA) grown in MEM media supplemented with 10 % FBS (Life Technologies, Grand Island, NY). Cells were harvested when they were at least 90 % confluent. An enriched mitochondria fraction was obtained from $2x10^7$ HTB-11 cells. Mitochondrial preparations were also obtained from $2x10^7$ white blood cells (WBC). Heparinized whole blood samples (50 mL per donor), from individuals without pre-existing pathophysiological conditions, were purchased from Long Island Blood Services (Westbury, NY) and immediately separated using 1-Step Polymorphs (Accurate Chemical and Scientific Corporation, Westbury, NY) gradient medium. Five ml of blood was layered over 5 ml of polymorphs in a 14 ml round-bottom tube and then centrifuged for 35 min at 500 g in a swinging-bucket rotor at 18 °C. After centrifugation, the bands consisting of polymorphonuclear cells (PMN) and mononuclear cells (MN) were harvested using glass pipettes and transferred into 15 ml centrifuge tubes and then washed with 10 ml phosphate buffered saline (PBS) (Invitrogen, Carlsbad, CA). Cells were collected after centrifugation for 10 min at 400 g. Cells were incubated at 37 °C in RPMI-1640 media supplemented with 10 % fetal bovine serum (Invitrogen) and allowed to recover for 60 min. WBC were washed and pelleted with cold (4 °C) PBS prior to mitochondrial isolation. The mitochondria were isolated using a mitochondria isolation kit for cultured cells (MITOISO2) (Sigma). This method results in an enriched intact mitochondrial fraction from cells. The detergent lysis method was used and required 650 μL of lysis buffer. The lysed cells were centrifuged at 700 g for 10 min and the resulting supernatant was centrifuged at 3000 g for 15 min. This next supernatant contained the cytosolic fraction and was transferred to another tube. The mitochondrial pellet was washed with 500 μL of the provided wash reagent. The washed mitochondria were centrifuged for 5 min at 12, 000 g. The supernatant was discarded and the pellet was re-suspended in 1 mL of storage buffer for use in NO determinations.

NO release from the mitochondria suspensions and from the cytosolic fraction of the mitochondrial isolation procedures were directly measured using a 700 μm flexible NO-specific amperometric probe (Innovative Instruments, Tampa, FL) connected to a 4-channel Biostat (ESA, Chelmsford, MA). The system was calibrated daily with S-nitroso-N-acetyl-DL-penicillamine in 0.1 M Cu^{2+}. The amperometric probe was allowed to equilibrate in storage buffer for at least 10 min prior to being transferred to the tube containing the mitochondria or cytosolic fractions. Morphine-stimulated NO release was evaluated at a final concentration of 10^{-7} M and 10^{-6} M. The receptor antagonist, naloxone (10^{-6} M), was added 10 min prior to morphine addition. N omega-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, was administered at $2X10^{-5}$M, 10 min prior to morphine addition. Ca^{2+} ($1X10^{-5}$ M) was also added to certain preparations of WBC mitochondria followed 60 s later by morphine (10^{-6}M). The detection limit of the NO probe was 0.5 nM. Each NO measurement was repeated six times with separately prepared mitochondria. All reagents were purchased from Sigma (St. Louis, Mo).

For protein quantification, mitochondria were lysed by vortexing for 1 min in 100 μL of 2 % CHAPS in Tris-buffered saline (25 mM Tris, 0.15 M NaCl; pH 7.2) and centrifuged at 12,000 g for 2 min. The supernatant was analyzed using the Bio-Rad protein assay and a microplate reader (Microplate Manager® 4.0). The cytosolic fractions from the mitochondrial isolation procedure were also assayed for their protein content and loaded into lanes next to the mitochondrial proteins. For Western blots, purified proteins (~1.4 μg per preparation) were combined with 10 μL of Bolt™ LDS sample buffer (4×) and 30 μL deionized water. Samples were denatured at 90 °C for 10 min and separated via protein electrophoresis on a NuPAGE® Novex® 4–12 % Bis-Tris Minigel at 165 V for 28 min. Following electrophoresis, the samples were transferred onto a nitrocellulose membrane using the iBlot® Gel Transfer Device system (Invitrogen). After that, using the iBlot® Gel Device for Western Detection (Invitrogen), the membrane was subjected to a 3-step program consisting of a blocking step, primary antibody step, and a secondary antibody step. The primary antibody, mouse anti-

Cytochrome C monoclonal, was obtained from Life Technologies (Invitrogen Cat. No. 33–8500) and diluted 1:1000 with blocking buffer. The secondary antibody was diluted 1:2000 with blocking buffer. The membrane was then sequentially washed three times in 1X wash solution and rinsed in deionized water before incubation at room temperature. Finally, with gentle rocking at room temperature the blot was developed using Chromogen (BCIP/NBT) as a chromogenic substrate.

Results

Pulsatile constitutive release of nitric oxide from purified mitochondrial preparations

As monitored by real-time amperometric analysis, suspensions of purified mitochondria prepared from cultured HTB-11 cells and pooled human leukocytes were observed to release NO into the extra-organelle medium over a defined time course (Fig. 1) . The constitutive mitochondrial membrane release of NO from both cellular preparations was temporally defined as a pulsatile function with a periodicity of approximately 4 s and peak amplitude of 1–2 nM (see Figs. 1, 2 and 3 prior to drug administration). The pattern of pulsatile constitutive NO release was observed to be highly reproducible based on 6–10 independently run preparations and appeared to be sustained over a relatively long time course of 20–30 min following dilution of purified mitochondrial preparations into extra-organelle release buffer.

Opiate dependence of nitric oxide release from purified mitochondrial preparations

As depicted in Fig. 1, addition of morphine sulfate to suspensions of purified HTB-11 mitochondrial preparations to achieve final concentrations of 10^{-7}M and 10^{-6} M, promoted transient increases in extra-organelle NO peak amplitude with 20 s latency over a short 20 s time course. Morphine sulfate additions resulted in 5 ± 1 and 12 ± 4 nM release of NO, respectively, (nM ± standard error of the mean, n = 6) (Table 1). The WBC preparations released comparable amounts of NO (Fig. 2). The 10^{-7} M morphine treatment released 9 ± 3 nM and the 10^{-6} M treatment resulted in 14 ± 4 nM NO (Table 1). Naloxone (10^{-6}M), the opiate receptor antagonist, inhibited morphine (10^{-7}M and 10^{-6}M) stimulated NO release (Fig. 3). L-NAME's (NOS inhibitor at 10^{-5} M) ability to inhibit morphine stimulated NO release confirmed that NOS was the enzyme producing NO and that it was constitutive (Fig. 3). Methionine enkephalin, an opioid peptide, did not stimulate NO release from mitochondria at any concentration (Fig. 4). Ca^{2+} added to the WBC mitochondrial pellet stimulated an NO production of 12 ± 4 nM and also increased the average amount of NO released by morphine addition to 28 ± 6 nM (Fig. 4 and Table 1). Importantly, the presence of cytochrome c (CYCS) immunoreactivity showed that the mitochondrial preparation contained mitochondrial membranes, validating the receptor's presence on mitochondria (Fig. 5). This was also validated by the fact it was absent from the cytoplasmic prep.

When challenged with 10^{-7} M morphine, HTB-11 cells usually release about 25 nM NO from $5X10^5$ cells (data not shown). Our HTB-11 mitochondrial preparation was from approximately $2X10^7$ cells. The initial release was calculated to be about 1 X 10^{-19} mol of NO per cell used in the mitochondrial preparation. We calculated that whole cells typically release $1X10^{-17}$ mol of NO per HTB-11 cell.

Discussion

The current data demonstrates, on both a pharmacological and physiological level, the presence of the 6 transmembrane opiate receptor subtype and its coupling to constitutive NO

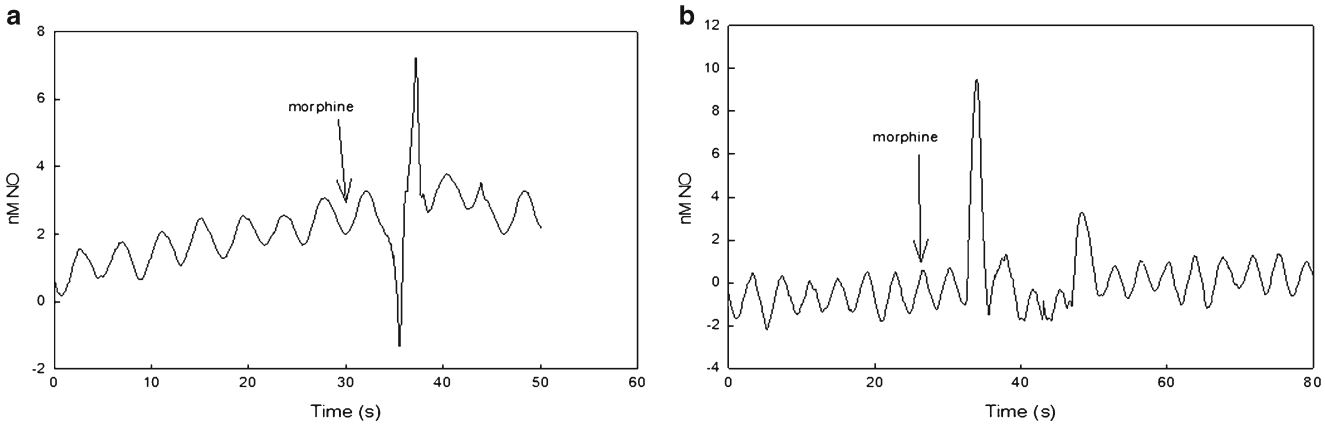

Fig. 1 Representative morphine (10^{-7} M and 10^{-6} M; left and right figures, Figures **a** and **b**, respectively) stimulated NO release from HTB-11 mitochondrial preparations (n = 6)

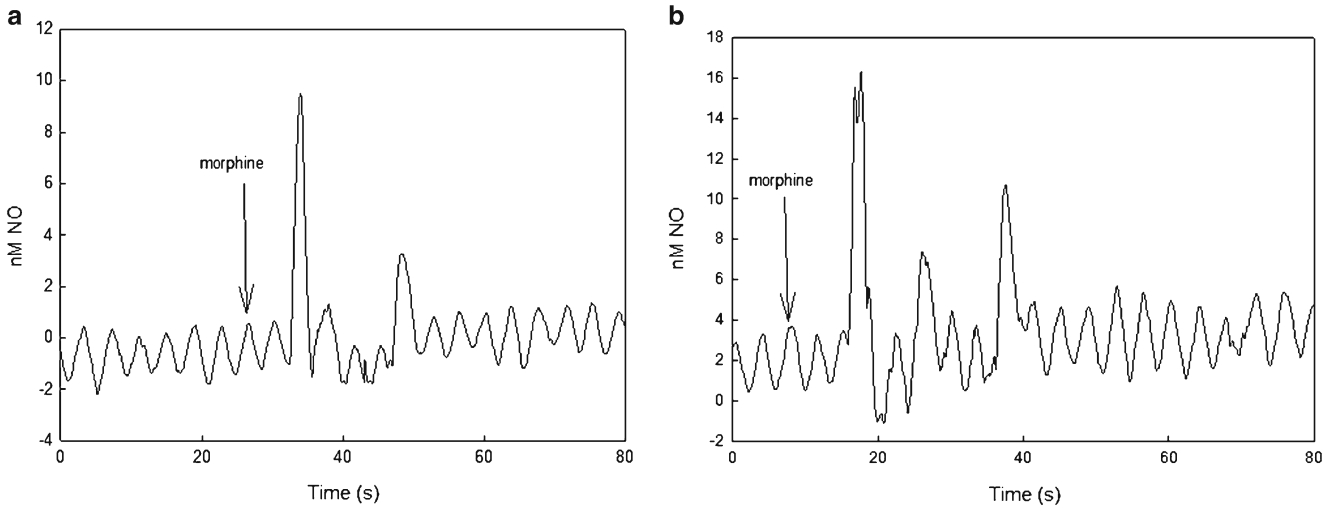

Fig. 2 Representative morphine (10^{-7} M and10^{-6} M; left and right figures, Figures **a** and **b**, respectively) stimulated NO release from WBC pellet mitochondrial preparation ($n = 5$)

release in mitochondrial membranes as opposed to cytoplasmic associated membranes. These results validate previous studies, which find evidence for cysteine clustering indicative of NO interaction within this receptor (Kream et al. 2007). In this regard, constitutive NO release is blocked by the opiate antagonist naloxone and the NOS inhibitor L-NAME, demonstrating it is receptor-mediated, and NOS coupled. Additionally, calcium enhances the level of the NO release, further identifying it as cNOS (Stefano et al. 2000a). Moreover, the identification of CYCS in the mitochondrial membrane preparation suggests the receptor is associated with the mitochondria. Besides these characteristics, the receptor-mediated process appears to be opioid peptide insensitive, providing further evidence of its identity as mu3- or mu4-like opiate receptor (Cadet et al. 2007).

Past research provides ample biochemical, pharmacological, and molecular evidence to support the existence and biological importance of two unique 6 transmembrane helical domain (TMH6) domain opiate receptors expressed from OPRM1 that are functionally coupled to Ca^{2+}-dependent production and release of NO from the constitutive isozyme of NO synthase (cNOS) (Cadet et al. 2003; Stefano 1998; Stefano et al. 1993; Stefano et al. 2000a; Stefano et al. 2000b; Stefano et al. 1995a; Stefano et al. 1996). Both receptors are Class A rhodopsin-like members of the superfamily of G-protein coupled receptors, but lack an amino acid sequence of approximately 90 amino acids that constitutes the extracellular N-terminal and TMH1 domains and part of the first intracellular loop of the mu 1 opioid receptor. This distinct protein modification confers highly selective binding and activation by morphine and morphinan-related BIQ alkaloids, but not by all classes of endogenous opioid peptides, due to retention of the essential ligand binding pocket distributed across conserved TMH2, TMH3, and TMH7 domains of the mu 1

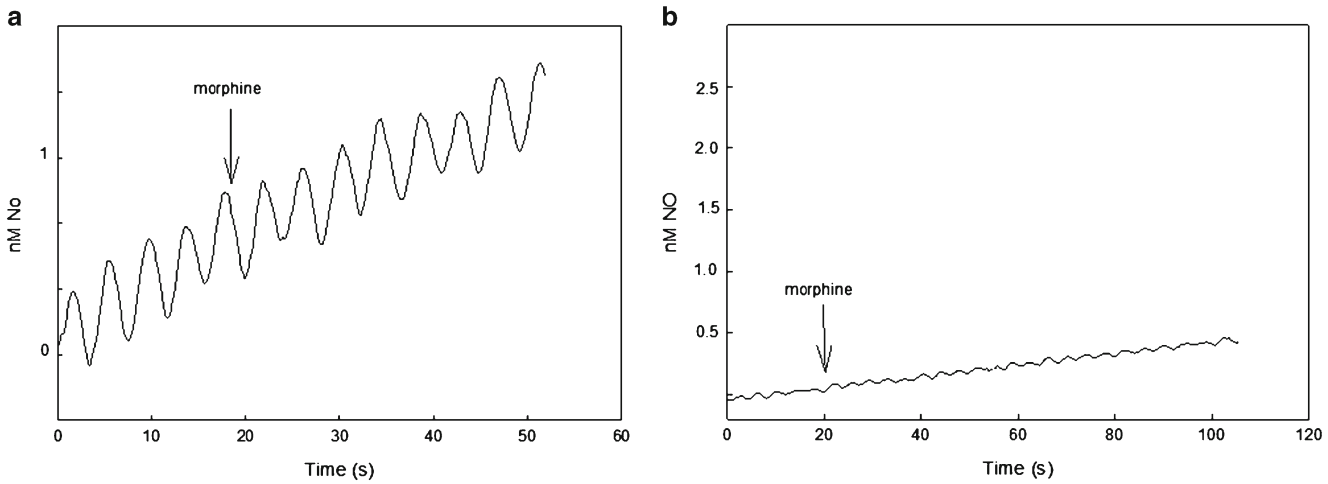

Fig. 3 Representative real-time NO readings. **a**. Left: Naloxone (10^{-6}M) blocks morphine-stimulated NO release from HTB-11 mitochondrial preparation. **b**. Right: L-NAME (10^{-4}M) antagonizes morphine-induced NOS stimulated the release of NO

Table 1 Nitric Oxide release from mitochondrial preparations ($n = 6$). Mitochondrial preparations were obtained from $2x10^7$ HTB-11 cells or $2x10^7$ WBC

Cell type (treatment)	nM NO ± SEM
HTB-11 (10^{-7} M morphine)	5 ± 1
HTB-11 (10^{-6} M morphine)	12 ± 4
WBC (10^{-7} M morphine)	9 ± 3
WBC (10^{-6} M morphine)	14 ± 4
WBC (10^{-5} M Ca^{2+})	12 ± 4
WBC (Ca^{2+} then morphine (10^{-6} M))	28 ± 6

sequence (Kream et al. 2007; Sheehan 2009). Thus, the six transmembrane opiate receptors are selectively tailored to mediate the cellular regulatory effects of endogenous morphine and related BIQ alkaloids within a highly restricted signaling pathway involving stimulation of NO production and release (Kream et al. 2007; Stefano et al. 2000a).

The biological significance of these novel, cognate, opiate receptors is supported by their expression by human multilineage progenitor stem cells (MPLC) in the absence of the traditional TMH7 domain mu 1 opioid receptor (Cadet et al. 2007). These data also suggest that these opiate receptors were probably the prototypes in the line of future μ, δ, and κ opioid receptor types. Structural analysis indicates that the unique cysteine cluster found at the C-terminal tail or intracellular domain of the mu 3 opiate receptor bears a striking sequence homology to similar cysteine clusters within the C-terminal domains of the CCR2B and CCR5 chemokine receptors (Venkatesan et al. 2001). The cysteine clustering represents a potential nitrosylation domain and docking site for covalent attachment to cNOS, further supporting the case for functional "morphinergic"/NO coupling as a key signal transduction mechanism (Kream et al. 2007). Finally, the ability of the 6 transmembrane opiate receptors to gate intracellular calcium transients, eventually affecting mitochondrial oxygen consumption and energy conservation (Table 2)(Stefano et al. 2012), provides a compelling functional linkage of these receptors with recently characterized calcium channels (Kotturi et al. 2006).

We have proposed that the expression of endogenous morphine by animal and human cells is designed to mediate homeopathic regulation of metabolic activity via activation of cognate 6 transmembrane opiate receptors that serve as transductive conduits for short-circuit Ca^{2+} fluxes (Fimiani et al. 1999; Kream and Stefano 2010; Nieto-Fernandez et al. 1999). Interactive regulatory pathways employing endogenously expressed morphine as an activating principle for Ca^{2+}-dependent, graded release of NO were fashioned as a key cellular signaling molecule, responsible for regulating intermediary metabolic functions, including mitochondrial respiratory rate (Table 2)(Kream and Stefano 2010; Stefano et al. 2001). Interestingly, the response to morphine stimulated NO release is associated with lowering the state of cellular excitability, exerting an inhibitory state (Stefano et al. 2000a; Stefano and Scharrer 1994).

The cardiovascular literature has provided us with a window of opportunity to investigate concerted regulatory activities of endogenous morphine and NO at the cellular and mitochondrial level (Stefano et al. 2001). First, in an ischemia-reperfusion rat model, administration of pharmacological dosages of morphine has been shown to reduce infarct size in the myocardium and promote improvement in cardiac function (Chang et al. 2005; Peart and Gross 2003). Additional studies have attributed the protective effects of morphine to the opening of mitochondrial K (ATP) channels in the myocardium (Chistiakov and Gegenava 1976; Chistiakov and Gegenava

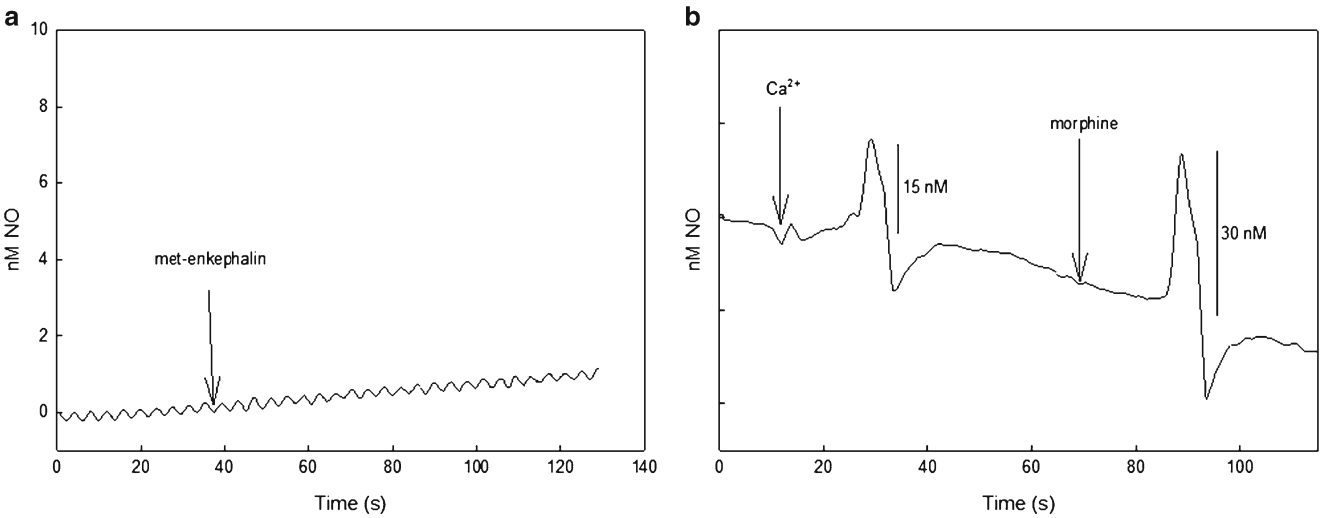

Fig. 4 The representative real-time action of opioid and opiate stimulated NO release in HTB cells. **a**. Left: The opioid peptide, met-enkephalin (10^{-5} M) did not stimulate NO release from HTB-11 mitochondrial membrane preparations at any concentration $10^{-6,-7}$ M tested. **b**. Right: Ca^{2+} stimulates NO release from mitochondrial preparations and enhances morphine (10^{-6} M) stimulated release

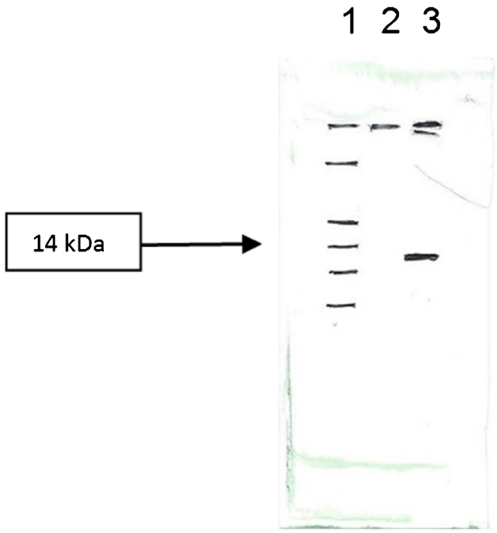

Fig. 5 Western blot for cytochrome c (expected size = 11.6 kDa). Lane 1 = ladder; lane 2 = cytoplasm protein; lane 3 = mitochondrial protein

1980; Cohen et al. 2001; Gegenava and Chistiakov 1975; Lishmanov et al. 2004; Ludwig et al. 2003; Maslov et al. 2003; McPherson and Yao 2001; Stefano et al. 2001). Subsequent studies have demonstrated that morphine protects the myocardium against ischemia-reperfusion injury via inhibition of glycogen synthase kinase-3beta (GSK3B) and its facilitation of mitochondrial permeability transition pore (mPTP) opening (Gateau-Roesch et al. 2006; Obame et al. 2008). Operationally, morphine protects the ischemic myocardium against Ca^{2+}-induced mPTP opening with subsequent increases in mitochondrial resistance and inactivation of GSK3B via phosphoinositide 3-kinase (PI3K) mediated events (Obame et al. 2008). A major cardio-protective effect of morphine is mediated through enhanced mitochondrial hexokinase binding (Zuurbier et al. 2009). The authors speculate that many cardio-protective interventions, including ischemic preconditioning and morphine administration during post conditioning, direct cellular redistribution and target mitochondrial hexokinase. Table 2 demonstrates morphine-mediated inhibition of mitochondrial-associated hexokinase, GSK3A, GSK3B, and cytochrome c oxidase gene expression in human MLPC.

In a parallel fashion to morphine, constitutive NO production and release protects the ischemic heart from apoptosis and mitochondrial dysfunction via protein kinase G-mediated blockade of mPTP opening and cytochrome c release (Borutaite et al. 2009). Furthermore, in a rat post-conditioning ischemia-reperfusion positive interactive effects of morphine and constitutive NO were observed (Jang et al. 2008). Extensive pharmacological controls using opiate receptor antagonists, cNOS and protein kinase inhibitors, provided validating evidence for selectivity of effect via concerted inhibition of mPTP opening by morphine and activation of the cNOS-protein kinase G pathway. A concerted pharmacological approach has recently been developed to selectively target NO donor compounds to mitochondria as an efficacious strategy to modulate respiration and protect mitochondria against ischemia-associated reperfusion injury (Prime et al. 2009). Prior studies have made the association between NO produced by a specific isotype found in the mitochondrion, mitochondrial NOS (mtNOS), in regulating cellular oxygen consumption/energy metabolism without engendering oxidative stress (Bates et al. 1996; Finocchietto et al. 2009; Giulivi et al. 2006; Kato and Giulivi 2006). Interestingly, older literature has observed opiate binding sites on rat liver mitochondria membranes (di Jeso et al. 1984; di Jeso et al. 1982) and noted its effects on oxidative phosphorylation. Additionally, a recently published report demonstrated the presence of mu 3 and mu 4-like opiate receptors in human cells and tissues (Andersen et al. 2013). Accordingly, homeopathic enhancement of endogenous morphine signaling in concert with mtNOS activation may represent a novel, non-invasive, strategy for maintaining myocardial integrity in normal and in pathophysiological conditions. Given the mitochondrial presence in all eukaryotic cells, abnormal cellular excitation may be targeted at this level with morphine. Morphine is an endogenous signaling chemical messenger, and we surmise that it may provide this activity in other disorders associated with abnormal mitochondrial activity (DiStefano and Paulesu 1994; Kream and Stefano 2010; Stefano and Scharrer 1994). This may also suggest that abnormal mitochondrial activity would emerge in an endogenous morphine insufficiency state.

Human white blood cells have the ability to make and release morphine (Zhu et al. 2005a), which can have autocrine and paracrine signaling functions (Fig. 6). In the case of autocrine via cell surface mu opiate receptors, nitric oxide would be released given its coupling to these mu opiate receptor subtypes (Cadet et al. 2003; Cadet et al. 2007; Casares et al. 2005; Kream et al. 2007; Zhu et al. 2005a). We have

Table 2 Down regulation of mitochondrial-associated gene expression, following morphine administration to human multi-lineage progenitor cells. Fold changes were calculated on raw signal values from microarrays. Fold changes at the −2.0 level represents a 100 % drop in values compared to controls. The presented values were obtained from previously data sets (Cadet et al. 2007) but not published in this form

Gene Symbol	Gene Name	Fold Change
HK3	hexokinase 3 (white cell)	−3.5
GSK3B	glycogen synthase kinase three beta	−1.8
GSK3A	glycogen synthase kinase three alpha	−1.9
GCKR	glucokinase (hexokinase 4) regulator	−1.8
COX7C	cytochrome c oxidase subunit VIIc	−2.0
COX7B	cytochrome c oxidase subunit VIIb	−2.0
COX6C	cytochrome c oxidase subunit VIc	−2.0
COX6B	cytochrome c oxidase subunit VIb	−2.0

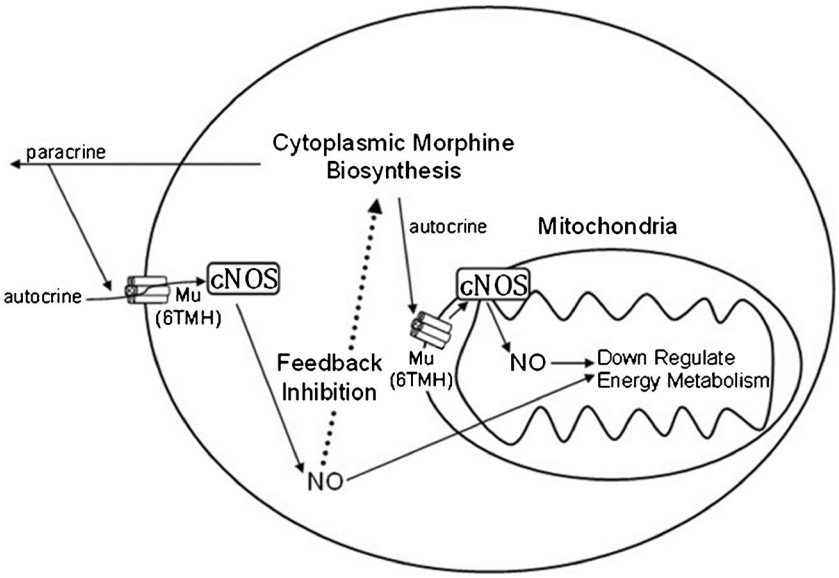

Fig. 6 Animal cells have the ability to make and release morphine. Additionally, this release can have autocrine and paracrine signaling functions via the novel six transmembrane helical domain mu opiate receptors (Mu TMH6) coupled to constitutive nitric oxide synthase (cNOS) activation. Simultaneously, morphine can negatively influence key mitochondrial energy associated enzymes and processes diminishing total energy metabolism. Given morphine's status as an endogenous signaling messenger, we surmise, that one of its functions is to regulate down excessive mitochondrial excitation via NO coupling. This, in part, explains its presence in animals and plants. (Kream and Stefano 2009; Stefano and Kream 2008)

demonstrated that the stimulated cellular NO can exert negative feedback actions on the enzymes that make morphine (Mantione et al. 2008). Simultaneously, it can negatively influence key mitochondrial energy associated enzymes and processes (Stefano et al. 2008), diminishing total energy metabolism (Giulivi et al. 2006) (Fig. 6). We surmise morphine signaling is protective because reactive oxygen species (ROS) and subsequent tissue damaging processes are diminished (Stefano and Scharrer 1994). Perhaps, it is this morphinergic influence that might have allowed the mitochondria to be enslaved as a cellular organelle, via diminishing its ability to generate uncontrolled ROS and energy associated phenomena detrimental to the host cell.

The present study adds to this mitochondrial modulation process by demonstrating the presence and identification of this receptor in this organelle as being opioid peptide insensitive and opiate alkaloid selective, placing opiate regulation directly in the mitochondria. Of equal significance, is the finding that a neuronal NOS, originally discovered in the cytoplasm, is also present in this organelle (Kanai et al. 2001) having the ability to produce measurable quantities of NO, which can also modulate mitochondrial energy pathways. NO can inhibit the reduction of oxygen to water by cytochrome c oxidase by competing for the enzyme's active sight (Brown and Cooper 1994; Giuffre et al. 2002; Kanai et al. 2001). Cytochrome c oxidase is thought to be protecting the mitochondria from excess NO (Sarti et al. 2003) by this reversible binding. This inhibition could lead to locally diminished ATP production until the NO is removed.

Conclusion

The widespread expression of morphine and its processes by plants and animals strongly indicates a high level of evolutionary conservation and focuses on its components as essential chemical mediators (Stefano and Kream 2007). Mitochondria arose via permanent enslavement of purple non-sulphur bacteria as endosymbionts (Cavalier-Smith 2006). In light of what has been presented above, primordial "morphinergic"/NO-coupled signaling may have been instrumental in the establishment of the mitochondrion as a viable eukaryotic organelle. Indeed, morphine-NO coupling is a potent inhibitor of cell motility across evolutionary lines via stabilization of cellular conformation (Stefano 1989; Stefano et al. 1995b; Stefano et al. 1994; Stefano and Scharrer 1994). Aberrant "morphinergic"/NO-coupled regulatory events at the mitochondrial level are proposed as causative factors in a variety of pathophysiological states that are associated with very basic metabolic dysfunction.

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