Review Article

Endogenous Morphine: Up-to-Date Review 2011

(endogenous morphine / dopamine / catecholamine / nitric oxide / nitric oxide synthase)

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Abstract. Positive evolutionary pressure has apparently preserved the ability to synthesize chemically authentic morphine, albeit in homeopathic concentrations, throughout animal phyla. Despite the establishment of a progressively rigorous and mechanistically focused historical literature extending from the mid 1970s to the mid 1980s that supported the expression of chemically authentic morphine by animal cellular and organ systems, prejudicial scepticism and early dismissal by scientists and clinicians most often obscured widespread acceptance of the biological importance and medical implications of endogenous morphine. The current critical paper presents and evaluates key recent coordinated studies in endogenous morphine research, highlighting those that have advanced our understanding of the functional roles of cognate alkaloid-selective μ_3 and μ_4 opiate receptors. We propose that the expression of endogenous morphine by animal and human cells is designed to mediate homeopathic regulation of metabolic activity via activation of cognate μ_3 and μ_4 receptors that serve as transductive conduits for short-circuit Ca^{++} fluxes. The implications of endogenous morphine coupling to nitric oxide regulation of mitochondrial function, with special reference to the cardiovascular system, are now formulated after many years of neglect.

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Abbreviations: BIQ – benzylisoquinoline, cNOS – constitutive NO synthase, COMT – catechol O-methyl transferase, CYP – cytochrome P450, DA – dopamine, DBH – dopamine β-hydroxylase, DDC – L-DOPA decarboxylase, DOPAL – 3,4-dihydroxyphenylacetaldehyde, GPCR – G-protein-coupled receptor, GSK-3β – glycogen synthase kinase 3β, IL – intracellular loop, L-DOPA – 3,4-dihydroxy-L-phenylalanine, L-TYR – L-tyrosine, MOR – μ opioid receptor, mPTP – mitochondrial permeability transition pore, mtNOS – mitochondrial NOS, NO – nitric oxide, NOS – nitric oxide synthase, PNMT – phenylethanolamine N-methyl transferase, ROS – reactive oxygen species, TA – tyramine, TH – tyrosine hydroxylase, THP – tetrahydropapaveroline, TMH – transmembrane helical, UCP2 – uncoupling protein 2, VT – volume transmission.

Historical perpectives

The burgeoning spectrum of empirical research into the biological roles of major families of endogenous opioid peptides and pharmacologically distinct types of opioid receptors rapidly attained a "high-profile" scientific status within the early to late 1970s (Kosterlitz and Hughes, 1977; Lord et al., 1977). As a corollary, a newly established scientific dogma elaborated plausible mechanistic schemes whereby pharmacological activities of a wide variety of opiate alkaloid drugs resembling the prototype narcotic analgesic morphine were mediated by distributions of opioid receptors within CNS and peripheral nervous structures that were normally responsive to activation by coordinated/regulated release of endogenous opioid peptides. In effect, morphine and its chemical congeners represented xenobiotic activating agents that with chronic usage brought about debilitating perturbations in the homeostatic integrity of endogenous opioid and functionally linked neural systems, thereby promoting dire behavioural consequences. From psychosocial, medical, and cultural perspectives, the double-edged sword of morphine's pain-killing properties inextricably linked to dependence and addiction would soon be put to rest by development of a new generation of opioid peptide analgesics.

Contemporaneously and tinged with a significant degree of irony, talented investigators working within the academic branch of a multi-national pharmaceutical company observed low steady-state levels of immunologically detectable morphine in several species of mammalian brain (Gintzler et al., 1976, 1978). Accordingly, the orphan discipline of endogenous morphine

research was born and during the next 10 years the Spector laboratory made considerable advances in characterizing biosynthetic events involving *in vivo* enzymatic conversion of morphinan precursors into endogenous morphine (Donnerer et al., 1986), notably the critical role of tissue cytochrome P450 (CYP) enzyme activity in these maturation processes (Kodaira and Spector, 1988). Essential cross-validation of these findings was accomplished by contributions from another prominent group of investigators who reported the presence of morphine-like and codeine-like immunoreactivities in mammalian nervous tissues that were chemically characterized as authentic morphine and codeine (Goldstein et al., 1985; Weitz et al., 1986, 1987), and demonstrated conversion of reticuline to salutaridine in rat liver, a critical step in generating the morphine/morphinan skeleton and the stereochemistry of the morphinan series (Weitz et al., 1987).

Despite the establishment of a progressively rigorous and mechanistically focused historical literature extending from the mid 1970s to the mid 1980s that supported the expression of chemically authentic morphine by animal cellular and organ systems, prejudicial scepticism and early dismissal by scientists and clinicians most often obscured widespread acceptance of the biological importance and medical implications of endogenous morphine. As a prime example, an evidence-based retrospective validation of the original report demonstrating well-defined discrete anatomical distributions, not random deposition, of morphine-like immunoreactivity within the mouse brain (Gintzler et al., 1978) utilizes collected pharmacokinetic data to eliminate back-door criticism contending that the observed effects resulted from either dietary sources or laboratory contamination (Kalvass et al., 2007). Following ingestion, plasma morphine is rapidly converted to the well-characterized morphine-3 and morphine-6 glucuronide metabolites within the liver. The hydrophilic properties of morphine-3 and morphine-6 glucuronides indicate that they do not readily penetrate the blood brain barrier. Because very little unconjugated morphine is actively transported into the brain, a local synthesis model is supported. Interestingly, this conclusion does not exclude, but in effect favours metabolic coupling of neurons and glia to explain endogenous morphine expression within the CNS.

Advanced biosynthetic studies

The veracity and medical ramifications of testable hypotheses relating to endogenous morphine expression by animal cells are highly dependent on strong unifying principles. Of prime importance is the establishment of extensive chemical identities between the elucidated morphine biosynthetic pathway in opium poppy (*Papaver somniferum*) with that of its animal system counterparts. Here elegant studies published by a leading plant science group in Canada have been invaluable (Facchini and De Luca, 1994, 1995; Facchini and Park, 2003). Dopamine (DA) is an essential precursor in the morphine biosynthetic pathway in Papaver and in the biosynthetic pathways of approximately 2500 chemically distinct benzylisoquinoline (BIQ) alkaloids expressed by plant orders Ranunculales, Eumagnoliids, Rutaceae, Lauraceae, Cornaceae and Nelumbonaceae (Liscombe et al., 2005; Liscombe and Facchini, 2008). The rate-limiting enzyme in all BIQ biosynthetic pathways is (S)-norcoclaurine synthase (NCS; EC 4.2.1.78). NCS catalyses a stereoselective Pictet-Spengler condensation and rearrangement utilizing the essential substrates DA and tyramine aldehyde to form norcoclaurine, the committed biosynthetic intermediate for all 2500 BIQ compounds including morphine. NCS activity has been demonstrated in at least 90 diverse plants species (Liscombe et al., 2005; Liscombe and Facchini, 2008) including the common meadow rue, *Thalictrum flavum* (Berkner et al., 2008).

The chemical characterization of an NCS-like enzyme in animal cells as of yet has not been definitely established. Prior neurochemical studies, however, have detected low concentrations of only the biologically relevant (S) enantiomer of the norcoclaurine homologue tetrahydropapaveroline (THP) in human brain, thereby indicating enzyme-catalysed expression of biosynthetic intermediates within a defined biosynthetic pathway (Sango et al., 2000). Demonstration of stereoselective expression of BIQ alkaloid precursors is complemented by later studies demonstrating the exclusive expression of the (S) enantiomer of the BIQ alkaloid morphine precursor (S)-reticuline in cultured SH-SY5Y human neuroblastoma and DAN-G human pancreatic carcinoma cells (Poeaknapo et al., 2004; Boettcher et al., 2005). Taken together, and complemented by studies of BIQ synthesis in *Papaver somniferum* (Samanani et al., 2004), kinetic criteria strongly suggest THP formation as a key regulatory/committed step in the biosynthetic pathway of endogenous morphine by animal cells (Kream and Stefano, 2006) (see Fig. 1).

Key observations from relatively recent studies performed in our laboratory indicate that L-tyrosine (L-TYR), its monoamine homologue tyramine (TA), and their respective catechol derivatives, 3,4-dihydroxy-L-phenylalanine (L-DOPA) and DA serve as substrates for *de novo* morphine production and that pharmacological characterization of TA utilization as a morphine precursor indicates one or more catalytic steps mediated by microsomal CYP 2D6 (Zhu et al., 2005a, b). The significance of TA as a biosynthetic intermediate is validated by *in vitro* enzyme kinetic studies demonstrating DA formation via CYP 2D6-catalysed ring hydroxylation of TA (Guengerich et al., 2002; Niwa et al., 2004), which in turn lends support to the existence of a potentially important TH-independent pool of cytosolic DA that is available for endogenous morphine expression (Zhu et al., 2005a, b, c). These data are complemented by metabolic labelling/isotope enrichment studies employing SH-SY5Y neuroblastoma cells (Poeaknapo et al., 2004; Boettcher et al., 2005), indicating asymmetric

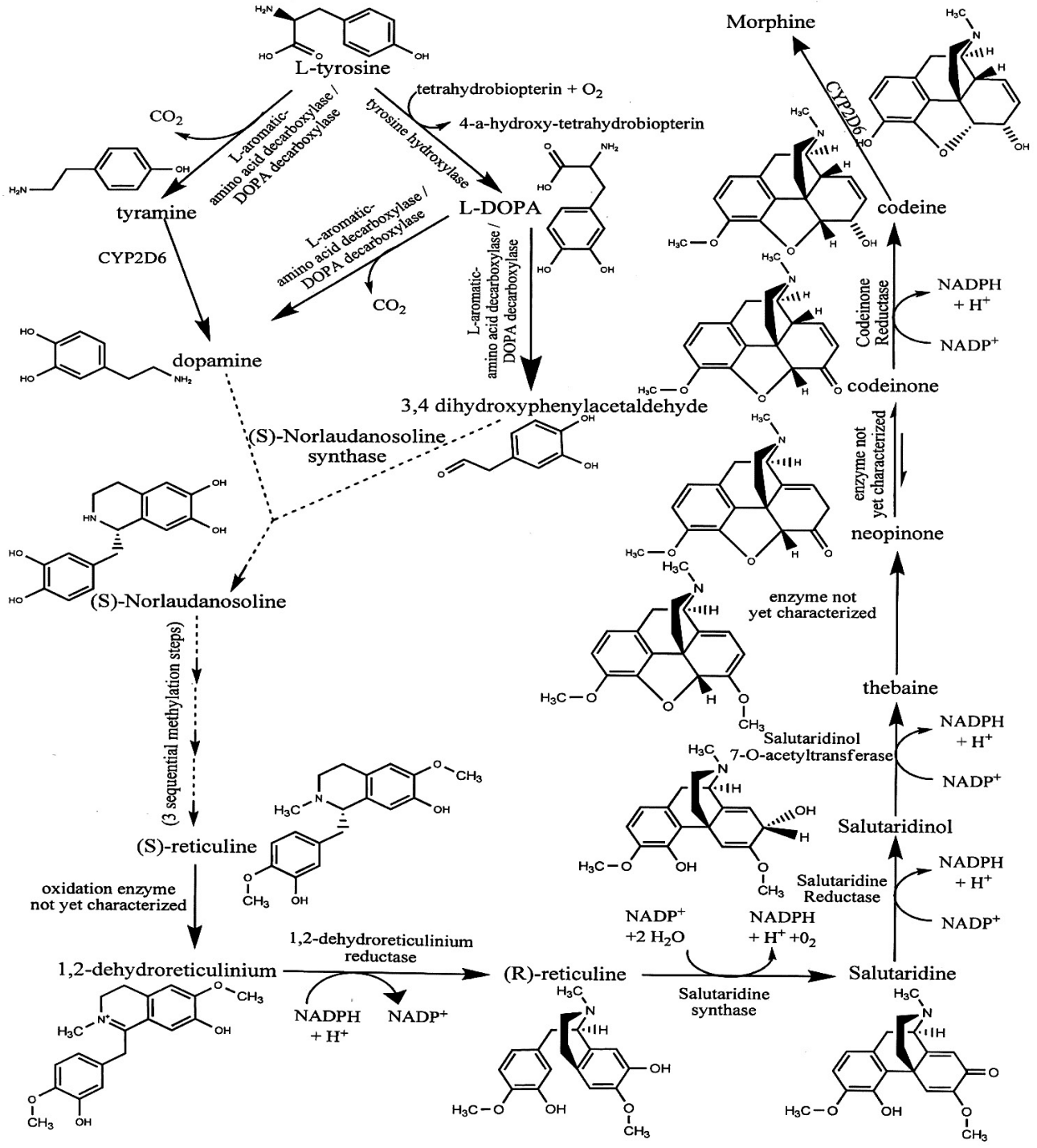

Fig. 1. De novo biosynthesis of morphine. With permission from Med. Sci. (Kream and Stefano, 2006).

isotopic labelling of the benzyl and isoquinoline chemical domains of newly formed morphine that is operationally determined by the type of L-TYR-derived precursor molecule that is employed: L-TYR and L-DOPA are incorporated in both the benzyl and isoquinoline chemical domains of morphine, whereas DA and TA are only incorporated into the isoquinoline domain (Fig. 1).

Collected data also effectively present a case for separate and distinct cellular pools of L-TYR-derived substrates targeted for *de novo* morphine synthesis in animal cells, and reject previously published biosynthetic schemes indicating that THP production is exclusively derived from DA (Kream and Stefano, 2006). THP formation involves enzymatic condensation and rearrangement of DA and 3,4-dihydroxyphenylacetaldehyde (DOPAL) (Cadet et al., 2007). Our formulated model establishes a *stoichiometric* relationship of one molecule of DA, derived from L-DOPA decarboxylase (DDC)-catalysed decarboxylation of L-DOPA or CYP2D6-catalysed ring hydroxylation of TA, to one molecule of DOPAL derived directly from L-DOPA without intermediate conversion to DA, thereby reject-

ing the long held hypothesis that MAO is a key enzyme involved in THP formation *in vivo* (Cadet et al., 2007).

Finally, the functional association of pyridoxal phosphate-dependent decarboxylases in opium poppy with mammalian DDC in the mediation of morphine biosynthesis in animal systems is compelling (Stefano and Kream, 2007). Accumulated biochemical data indicate that in addition to its well-characterized decarboxylation of L-aromatic amino acids, as a side reaction mammalian DDC catalyses a decarboxylation-dependent transamination or oxidative deamination of aromatic amines, effectively converting L-DOPA into DOPAL with simultaneous conversion of enzyme-bound pyridoxal phosphate into pyridoxamine phosphate (Zuurbier et al., 2009). The reaction specificity of DDC toward aromatic amines depends on the experimental conditions: oxidative deamination occurring under aerobic, half-transamination occurring under anaerobic conditions. Morphine biosynthesis in animals must include regulatory mechanisms responsible for the compartmentalization and mobilization of essential substrate pools of L-TYR and L-TYR-derived molecules targeted for BIQ alkaloid production. We propose reversible transamination of L-TYR and/or L-DOPA via pyruvic acid intermediates as a major regulatory mechanism responsible for cellular sorting and/or functional sequestration of substrate pools of L-TYR-derived molecules targeted for endogenous morphine production (Fig. 1).

Cognate 6 transmembrane helical domain μ opiate receptors functionally coupled to nitric oxide signalling

The apparently serendipitous finding of an opiate alkaloid-sensitive, opioid peptide-insensitive, μ_3 opiate receptor subtype expressed by invertebrate immunocytes, human blood monocytes, macrophage cell lines, and human blood granulocytes provided compelling validating evidence for an autonomous role of endogenous morphine as a biologically important cellular signalling molecule (Stefano et al., 1993; Cruciani et al., 1994; Stefano and Scharrer, 1994; Makman et al., 1995). More recent studies have provided biochemical, molecular, and pharmacological characterization of two unique six-transmembrane helical (TMH) domain opiate receptors expressed from the μ opioid receptor (MOR) gene (Stefano and Kream, 2007). Designated μ_3 and μ_4 receptors, both protein species are Class A rhodopsin-like members of the superfamily of G-protein-coupled receptors (GPCRs) but are selectively tailored to mediate the cellular regulatory effects of endogenous morphine and related morphinan alkaloids via stimulation of nitric oxide (NO) production and release (Fig. 2). Both μ_3 and μ_4 receptors lack an amino acid sequence of approximately 90 amino acids that constitute the extracellular N-terminal and TMH1 domains and part of the first intracellular loop (IL) of the μ_1 receptor, but retain the empirically defined ligand-binding pocket distributed across conserved TMH2, TMH3, and TMH7 domains of the μ1 sequence (Kream et al., 2007). Additionally, the receptor proteins are terminated by unique intracellular C-terminal amino acid sequences that serve as putative coupling or docking domains required for constitutive NO synthase (cNOS) activation. Because the recognition profile of μ_3 and μ_4 receptors is restricted to rigid BIQ/morphinan alkaloids typified by morphine and its extended family of chemical congeners, we hypothesized that conformational stabilization provided by interaction of extended extracellular N-terminal protein domains and the extracellular loops is required for binding of endogenous opioid peptides as well as synthetic flexible opiate alkaloids.

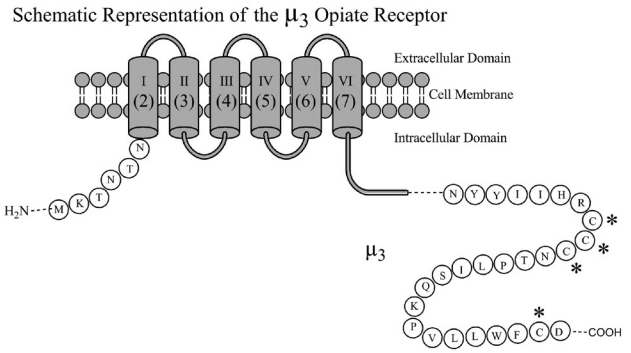

Fig. 2. Schematic representation of six TMH μ opiate receptors. TMH domains of μ_3 and μ_4 receptors are numbered I–VI and correspond to conserved TMH domains 2–7 of the μ_1 receptor. Conserved ELs, ILs, and C-terminal intracellular sequences common to μ_1, μ_3 and μ_4 receptors are represented by thick grey lines. Unique C-terminal intracellular domains of μ_3 and μ_4 receptors are represented by the single letter amino acid code. The conserved intracellular N-terminus of μ_3 and μ_4 receptors expressed from Exon 1 of the μ receptor gene is represented by the single letter amino acid code. With permission from Med. Sci Monit. (Kream et al., 2007).

Cellular expression of "truncated" six-TMH domain opiate receptors had been previously alluded to by molecular studies of μ receptor-encoding mRNA splice variants (Fig. 2). These accumulated data suggest that μ_3 and μ_4 receptors may be representative members of a "budding" family of primordial GPCR responsible for autocrine/paracrine regulation of cellular metabolic activity via local circuit Ca^{++} gating and NO feedback inhibition (Kream et al., 2007). In effect, μ_3 and μ_4 receptors represent cognate novel GPCRs mediating signalling events within a short loop "morphinergic" regulatory pathway activated by endogenous morphine and naturally expressed active morphine congeners such as its 6-glucuronide conjugate and functionally coupled to cNOS.

We have hypothesized that the expression of endogenous morphine by animal and human cells is designed to mediate homeopathic regulation of metabolic activity via activation of cognate μ_3 and μ_4 receptors that serve as transductive conduits for short-circuit Ca^{++} fluxes

(Stefano and Kream, 2008, 2010; Kream and Stefano, 2009, 2010; Kream et al., 2009, 2010; Mantione et al., 2008; Stefano et al., 2008a, b). The profound implications of our recent demonstration of a μ_4 receptor/NO-coupled regulatory pathway in human MLPC indicate that comparative phylogenetic analysis of the μ receptor gene may provide answers as to whether six-TMH domain μ_3 and μ_4 receptors are prototypic evolutionary models that have given rise to seven-TMH domain μ, δ, and κ receptors (Cadet et al., 2007). We have also hypothesized that the primordial "morphinergic" signalling pathway served as a prototypic model by which diverse catecholamine signalling pathways were formulated. Furthermore, it appears that the unique cysteine cluster found at the C-terminal tail or intracellular domain of the μ_3 opiate receptor bears a striking sequence homology to similar cysteine clusters within the C-terminal domains of the CCR2B and CCR5 chemokine receptors (Kream et al., 2007). In the case of CCR5, mutational analysis demonstrates that receptor function is critically linked to maintaining the integrity of the intracellular cysteine residues. Additionally, the cysteine cluster on the C-terminal tail of the μ_3 receptor represents a potential nitrosylation domain as well as a docking site for covalent attachment to cNOS, further supporting the case for functional coupling of these signal molecule systems (Kream et al., 2007). Similar criteria are presumably applicable to structure/function relationships of the μ_3 opiate receptor and establish evolutionary linkages between opiate and chemokine signalling processes early during evolution. Finally, recent provocative studies have probed the functional role of the toll-like receptor 4 (TLR4) in opioid-induced glial activation and recruitment of proinflammatory mediators at the spinal level. The authors conclude that TLR4 signalling involves non-classical opiate activation subsequent to xenobiotic alkaloid exposure (Kim et al., 2006). Studies designed to evaluate the putative interactive role of TLR4 signalling in relation to ongoing μ_3 and μ_4 receptor activation by endogenous morphine may shed light on normal neuronal-glial coupling events.

Functional coupling to dopamine systems

Dopamine serves as an obligate chemical intermediate in morphine and BIQ biosynthesis across plant orders without assuming an independent role as a prototype catecholamine signalling molecule (Fig. 1). Accordingly, we have formulated a hypothesis stating that catecholamine-expressing signalling systems emerged from the morphine biosynthetic pathway via evolutionary adaptation of key enzymes involved in the modification of L-TYR, DA, L-DOPA, and TA (Kream and Stefano, 2006). Notably, the plant N-methyl and O-methyl transferases required for conversion of the essential morphine precursor THP to the pre-morphinan alkaloid S-reticuline have been adaptively transformed into major enzymes in catecholamine expression, i.e. phenylethanolamine N-methyl transferase (PNMT) and catechol O-methyl transferase (COMT), respectively. Accordingly, evolutionarily driven chemical modifications of DA necessary for the cellular expression and utilization of epinephrine as a neural/neuroendocrine signalling molecule required co-ordinate recruitment and complex regulation of PNMT within tyrosine hydroxylase (TH)- and dopamine β-hydroxylase (DBH)-positive cells (Stefano and Kream, 2007). Finally, it is likely that TH preceded DBH in the evolutionary scheme, reflecting the appearance of norepinephrine in select long-lived invertebrates that required a higher level of motor-associated mobilization strategies (Stefano and Kream, 2007).

From a medical perspective, the functional association between aberrant DA metabolism, cellular expression of alkaloids, i.e., THP, and the aetiology of Parkinson's disease has been extensively studied and debated. It was subsequently demonstrated that urinary levels of morphine, codeine, and THP in L-DOPA-treated Parkinsonian patients are dramatically elevated (Kream and Stefano, 2006). The enhanced production of THP in Parkinsonian patients was peremptorily linked to the mediation of adverse side effects and cellular toxicity evolving from chronic L-DOPA therapy, despite clinical evidence supporting positive effects of morphine on L-DOPA-associated dyskinesias. The production of THP and endogenous morphine in Parkinson's disease patients undergoing chronic L-DOPA therapy provides strong presumptive evidence in support of recent *in vitro* biosynthetic studies utilizing L-DOPA as a key substance in *de novo* expression of morphine by diverse animal systems.

Finally, recent advances in Volume Transmission (VT) theory relate local temperature gradients created by brain uncoupling protein 2 (UCP2) to enhanced diffusion and convection of DA and opioid peptides in discrete CNS regions exhibiting transmitter-receptor mismatches. As presented in the following section, endogenous morphine and NO signalling mediate profound regulatory effects on mitochondrial function. Accordingly, studies to evaluate the functional role of endogenous morphine on VT in DA-ergic brain regions are potentially fraught with biological importance (Fuxe et al., 2005).

Mitochondrial targeting and the cardiovascular system

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 μ_3 and μ_4 receptors that serve as transductive conduits for short-circuit Ca^{++} fluxes (Fig. 3). Interactive regulatory pathways employing endogenously expressed morphine as an activating principle for Ca^{++}-dependent, graded release of NO were fashioned as a key cellular signalling molecule responsible for regulating intermediary metabolic functions, including mitochondrial respiratory rate.

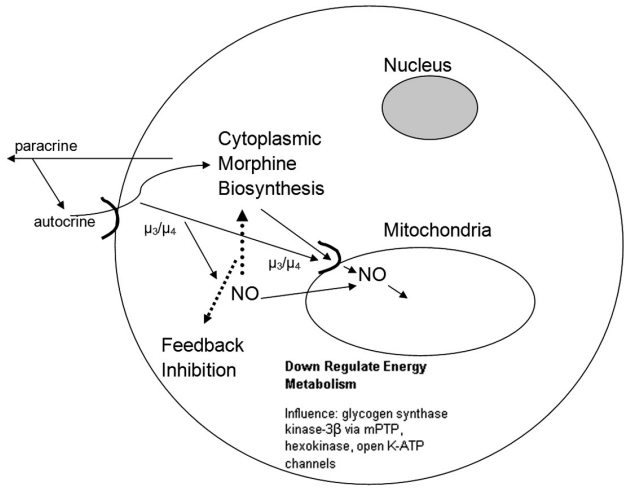

Fig. 3. Human white blood cells have the ability to make and release morphine, which can have autocrine and paracrine signalling functions. In the case of autocrine via cell surface μ_3/μ_4 receptors NO would be released given its coupling to these μ opiate receptor subtypes. Additionally, μ opiate receptors, as noted in the text, are on mitochondrial membranes. We have demonstrated that the stimulated cellular NO can exert negative feedback actions on the enzymes that make morphine and simultaneously negatively influence key mitochondrial energy-associated enzymes and processes, as noted in the figure, which when taken in total diminishes energy metabolism. In so doing we surmise reactive oxygen species (ROS) and tissue-damaging processes will be diminished; thus, morphine exerts an overall protective effect. We also surmise it is this morphinergic influence that may allow the mitochondria to be enslaved as a cellular organelle via diminishing its ability to generate uncontrolled ROS and energy-associated phenomena detrimental to cells. With permission from Med. Sci. (Kream and Stefano, 2009).

The cardiovascular literature has provided us with a window of opportunity to investigate concerted regulatory activities of endogenous morphine and NO at the cellular level (Banach et al., 2010). First, in an ischaemia-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 (Stefano et al., 2001). Additional studies have attributed the protective effects of morphine to the opening of mitochondrial K(ATP) channels in the myocardium (Stefano et al., 2001) (Fig. 3). Subsequent studies have demonstrated that morphine protects the myocardium against ischaemia-reperfusion injury via inhibition of glycogen synthase kinase 3β (GSK-3β) and its facilitation of mitochondrial permeability transition pore opening (mPTP). Operationally, morphine protects the ischaemic myocardium against Ca^{++}-induced mPTP opening with subsequent increases in mitochondrial resistance and inactivation of GSK-3β via PI3-kinase-mediated events. A recent review has indicated that a major cardio-protective effect of morphine is mediated through enhanced mitochondrial hexokinase binding (Kream and Stefano, 2009). The authors speculate that many cardio-protective interventions, including ischaemic preconditioning and morphine administration during postconditioning, direct cellular redistribution and target mitochondrial hexokinase.

In a parallel fashion to morphine, constitutive NO production and release protects the ischaemic heart from apoptosis and mitochondrial dysfunction via protein kinase G-mediated blockade of mPTP opening and cytochrome c release. Furthermore, in a rat postconditioning ischaemia-reperfusion model interactive positive effects of morphine and constitutive NO were observed. Extensive pharmacological controls using opiate receptor antagonists, cNOS and protein kinase inhibitors, provided validating evidence for selectivity of the 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 ischaemia-associated reperfusion injury. Prior studies have made the association between NO produced by a specific isotype found in the mitochondrion, i.e., mitochondrial NOS (mtNOS), in regulating cellular oxygen consumption/energy metabolism without engendering oxidative stress. Interestingly, older literature had observed opiate-binding sites on rat liver mitochondria membranes. Accordingly, homeopathic enhancement of endogenous morphine signalling in concert with mtNOS activation may represent a novel, non-invasive, strategy for maintaining myocardial integrity in normal and in pathophysiological conditions.

Concluding thoughts

Papaver somniferum synthesizes morphine as a major phytoalexin against microbial insult. The mythic and medically-defined properties of morphine as a double-edged pharmacological principle have often obscured its basic biological role as a plant alkaloid devoted to antimicrobial host defence. Recent empirical findings have contributed valuable mechanistic information in support of a regulated *de novo* biosynthetic pathway for chemically authentic morphine in eukaryotic cells, with many similarities to the extensively characterized multi-enzyme plant pathway, in concert with current advances in phytoalexin research, i.e., the study of plant-derived natural products in relation to their biological targets (Bednarek and Osbourn, 2009; Burdon and Thrall, 2009).

We are now in a facilitated position to elucidate the role of endogenous morphine in animal cell function. The widespread expression of morphine by plants, invertebrate and vertebrate cells/organ systems strongly indicates a high level of evolutionary conservation of morphine and related morphinan alkaloids as essential chemical factors required for normal growth and development.

Endogenously expressed, chemically authentic, morphine was fashioned as a key cellular signalling molecule, responsible for regulating intermediary metabolic functions, including mitochondrial respiratory rate. Evolutionary pressure was instrumental in forming functional links between cellular/tissue activation, inhibitory tone, nociception, and antinociception. This primarily involved multiple survival mechanisms by which primordial/progenitor cell types obtained the capability to regulate their responsiveness to environmental threats with minimal perturbations of metabolic homeostasis. Accordingly, nature has provided a multipurpose chemical messenger/protein modifier in the form of the free radical gas NO. Cells that emerged with the ability to temporally recruit and regulate NO expression, ostensibly via endogenous morphine coupling, within discrete microdomains possessed a major survival strategy that has been sustained throughout the course of evolutionary adaptations.

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