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Interactions between morphine and nitric oxide in various organs

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Abstract
Nitric oxide (NO) plays obligatory roles as an important intercellular messenger in the control of physiological functions and it also participates in pathophysiological interventions. This labile, gaseous molecule is also involved in mechanisms underlying the beneficial and untoward actions of therapeutic agents. Endogenous NO is formed by endothelial and neurogenic NO synthases that are constitutively present mainly in the endothelium and nervous system, respectively, and is induced by lipopolysaccharides or cytokines mainly in mitochondria, glial cells, and vascular smooth muscle cells. NO modulates the effects of morphine on processes involving the central nervous system, such as learning, memory, convulsion, thermoregulation, and penile erection. This molecule is also involved in the modification of morphine actions on the cardiovascular, digestive, and respiratory systems. Morphine regulates NO bioavailability in various organs. NO formed by inducible NO synthase participates in some morphine actions in the immune system. Information concerning interactions between NO and morphine and other opioids in a variety of organs and tissues is quite useful in establishing new strategies for minimizing the noxious and unintended reactions that are frequently encountered during analgesic therapy.

Key words Morphine · Nitric oxide · Cardiovascular system · Digestive system · Respiratory tract

Introduction

Nitric oxide (NO) is widely recognized as an intercellular messenger that plays important roles in the regulation of physiological functions and, in contrast, also participates in pathophysiological interventions. Impairment of NO generation or action and impairment of the downstream guanylyl cyclase/cyclic guanosine monophosphate (cyclic GMP) pathway is involved in the pathogenesis of various diseases, such as atherosclerosis, hypertension, coronary vasospasm, cerebral infarction, diabetes mellitus, gastrointestinal disorders, and erectile dysfunction. The NO/cyclic GMP signaling pathway contributes to mechanisms underlying the action of therapeutic agents, the most promising therapeutic mechanisms occurring through enhancing the action of endogenous NO via phosphodiesterase-5 inhibitors [1,2]. Some literature on opioid analgesic research has revealed that NO is involved in therapeutic actions, but that it can have untoward effects. Information concerning interactions between NO and morphine in central and peripheral organs would provide us with clues for establishing a strategy for minimizing the side effects of opioid therapy.

Our recent review article [3] has described how morphine-induced analgesia, tolerance, dependence, and the morphine withdrawal syndrome are modulated by morphine's interaction with NO. The present article describes interactions between NO and morphine in the central and autonomic efferent nervous systems and in the cardiovascular, digestive, respiratory, and immune systems.

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Synthesis, degradation, and actions of nitric oxide

NO is produced when L-arginine is transformed to L-citrulline by the catalysis of NO synthase (NOS) in the presence of O_2 and cofactors. Ca^{2+} is required for the activation of neuronal and endothelial NOS (nNOS and eNOS), but not inducible NOS (iNOS). nNOS is constitutively expressed in the brain [4], peripheral nerves, and kidneys, and eNOS is constitutively expressed mainly in endothelial cells [5]. iNOS is not constitutively expressed, but is induced mainly in macrophages by bacterial lipopolysaccharide (LPS) and cytokines. The synthesis of NO by these NOS isoforms is inhibited by L-arginine analogs, including N^G-monomethyl-L-

arginine (L-NMMA) [6], N^G-nitro-L-arginine (L-NA) [7,8], L-NA methylester (L-NAME) [7], and asymmetric dimethylarginine (ADMA) [9]. 7-Nitroindazol (7-NI) is a most promising nNOS inhibitor so far introduced [10] and aminoguanidine has been regarded as a selective iNOS inhibitor [11]. Nitro compounds, such as nitroglycerin and sodium nitroprusside (SNP), are capable of liberating NO.

NO or nitrovasodilators activate soluble guanylyl cyclase and produce cyclic GMP from guanosine triphosphate (GTP). Methylene blue, oxyhemoglobin, and 1H[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) [12] decrease the synthesis of cyclic GMP by inhibiting guanylyl cyclase activity. Cyclic GMP is degraded by phosphodiesterase type-5 to 5′-GMP.

Endothelial NO causes vasodilatation, increased blood flow, lowered blood pressure, inhibition of platelet aggregation, and inhibition of leukocyte adhesion. Nonadrenergic noncholinergic inhibitory responses to autonomic nerve stimulation are mainly mediated through NO synthesized by nNOS; NO plays a crucial role as a neurotransmitter from the peripheral efferent nerves in blood vessels [13], the gastrointestinal tract [14,15] the broncho-tracheal tract [16], and the corpus cavernosum [17]. NO signaling appears to be essential for neural plasticity; that is, long-term potentiation in the hippocampus and long-term depression in the cerebellum. NO formed by *N*-methyl-D-aspartate (NMDA) receptor activation diffuses to adjacent nerve terminals to modulate neurotransmitter release [18].

Under pathological conditions (e.g., during inflammation), high levels of NO are produced after the induction of iNOS expression, mainly in macrophages. On the one hand, NO exerts beneficial effects by acting as an antibacterial, antiparasitic, or antiviral agent, or as a tumoricidal agent; on the other hand, high levels of NO, if uncontrolled, elicit detrimental effects that are produced because NO reacts with concomitantly produced superoxide anions, thereby generating highly toxic compounds, such as peroxynitrite.

Central nervous system (CNS)

Ca^{2+}-dependent NOS in cerebellum was increased in mice treated with morphine, and the effect was blocked by coadministration of naloxone [19]. Morphine was able to stimulate NO release from rat hippocampus and amygdalar tissues in a naloxone- and L-NAME-sensitive manner [20]. The number of nNOS-positive neurons was higher in the hippocampal dentate gyrus of mice treated with either morphine or cocaine than the number of such neurons in saline-treated mice [21] In contrast, Dyuizen et al. [22] noted that the administration of morphine suppressed nicotinamide adenine dinucleotide phosphate, reduced nicotinamide adenine dinuceotide phosphate (NADPH) diaphorase-positive (NO-synthesizing) neurons in rat brain cervical nuclei, and naloxone reversed the morphine actions.

Learning, memory, and behavior

Post-training administration of L-arginine facilitated and post-training administration of L-NAME impaired memory consolidation in mice, but the pretest injection of either compound had no effect on retention; post-training administration of L-arginine at the noneffective doses reversed the impairment of memory formation induced by morphine, suggesting that the morphine-induced impairment involved decreased synthesis/release of NO and could be counteracted by a NOS substrate [23] (Fig. 1). L-Arginine, but not L-NAME, induced self-administration behavior and increased locomotion in rats; acute and chronic administration of L-arginine reduced morphine self-administration, while L-NAME increased morphine self-administration [24]. NO may have a role in morphine self-administration. Pretraining administration of morphine decreased the learning of a one-trial passive avoidance task in mice, pretraining administration of L-arginine alone did not affect memory formation, but it decreased the amnesia induced by pretraining with morphine, and the pretraining administration of L-NAME impaired memory formation; pretraining administration of apomorphine, a dopaminergic receptor agonist, inhibited the morphine-induced amnesia, whereas the inhibition of the

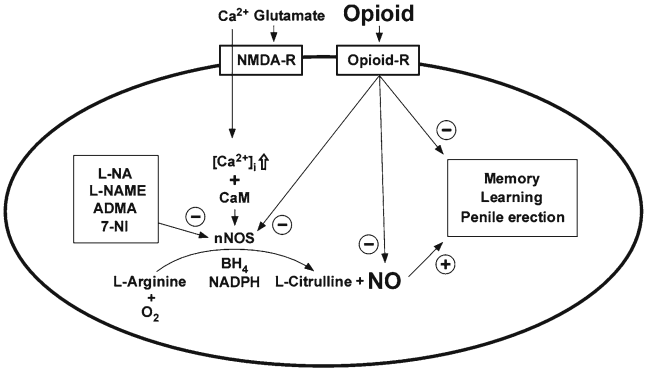

Fig. 1. Possible mechanisms of central action of opioids through NO bioavailability on memory, learning, and penile erection. The inhibitory effects of opioids on NO synthesis and actions impair the stimulating effect of NO on memory, learning, and penile erection. *(+)*, Stimulation; *(–)*, inhibition; *opioid R*, opioid μ-receptor; *NMDA-R*, *N*-methyl-D-aspartate (NMDA) receptor; *CaM*, calmodulin; *L-NA*, N^G-nitro-L-arginine; *L-NAME*, L-NA methylester; *ADMA*, asymmetric dimethylarginine; *7-NI*, 7-nitroindazol; *nNOS*, neuronal NO synthase; *NADPH*, nicotinamide adenine dinucleotide phosphate reduced; *[Ca2+]i*, intracellular Ca^{2+}

morphine-induced amnesia by L-arginine was decreased by pretreatment with a dopamine D_1- or D_2-receptor antagonist [25]. It appears that the morphine-induced impairment of memory formation is prevented by NO, and that a dopaminergic mechanism is involved in this effect. Morphine inhibited the K^+-stimulated release of [^{3}H]dopamine in a naloxone- and L-NAME-sensitive manner in the ventral chain ganglia of the leech *Hirudo medicinalis* and the pedal ganglion of the mussel *Mytilus edulis* [26]. Whether morphine-induced amnesia is counterbalanced by a decreased release of dopamine has not been determined.

L-Arginine and the NO donor S-nitroso-N-acetylpenicillamine (SNAP) reduced the anxiolytic effect of morphine in the plus-maze, and L-NA and L-NAME enhanced the effect; the morphine-induced locomotor activity was increased by L-arginine and SNAP, decreased by L-NAME, and not changed by L-NA [27]. The anxiolytic effects of morphine seem to be modulated by NO systems. Intrathecal administration of morphine to rats elicited excitatory behavioral syndrome and elevated extracellular glutamate and NO metabolites in the spinal cord; pretreatment with N-terminal fragment substance P (1–7) reversed the behavioral excitation and the increased glutamate and NO metabolites evoked by morphine [28]. The inhibitory effects of substance P (1–7) may be mediated by attenuation of the presynaptic release of glutamate and the reduction of NO production in the spinal cord.

On the other hand, opioids increase dopaminergic turnover in the nucleus striatum and nucleus accumbens of mice, causing behavioral changes. L-Arginine administration increased morphine-induced locomotion and changes in food intake in mice, and in contrast, L-NAME reduced the effects of morphine [29]. Endogenous NO may play a role in the modulation of the dopaminergic effects elicited by morphine. The administration of L-arginine or L-NAME before training did not alter morphine state-dependent learning; during the acquisition of sensitization, L-arginine administered before morphine increased, while L-NAME administered before morphine decreased morphine-state dependency, suggesting that NO is involved in the morphine-induced learning sensitization [30]. Whether the controversial effects of endogenous NO, that it either enhances or inhibits morphine actions, are associated with low and high concentrations of NO formed by cNOS and iNOS, respectively, remain to be determined. Human neuroblastoma cells are capable of synthesizing morphine [31]. In human neuroblastoma cells, Pak et al. [32] showed that morphine downregulated the expression of β-site amyloid precursor protein cleaving enzyme-1 (BACE-1), and upregulated the expression of BACE-2 in a naloxone-antagonizable manner, and treatment with L-NAME blocked the effects of morphine; SH-SY5Y cells treated with β-amyloid peptide showed a decrease in NO release. These authors suggested that a deficiency in the basal release of NO or endogenous morphine may trigger drastically reduced levels of basal NO, resulting in chronic vasoconstriction, brain hypoperfusion, and neuronal cell death. Based on these observations, the authors postulated a vascular pathological hypothesis for Alzheimer's disease.

The μ3 opiate receptors stimulated by morphine are coupled to cNOS-derived NO release; constitutive NO signaling is surmised to be critical in the relaxation response [33,34], which is defined as being the opposite of the stress response [35]. Stefano et al. [36] have summarized the view that NO and morphine control catecholamine processes on many levels, including the synthesis, release, and actions of catecholamine, and they have hypothesized, in light of the current understanding of central and peripheral nervous system mechanisms, that constitutive NO signaling is critical in relaxaiton.

Conditioned place preference

L-NA, given intraperitoneally, inhibited morphine-induced place preference in rats, but L-NA by itself showed no reliable effect on place conditioning, suggesting a possible role of NO in the opioid reward process [37]. Intrahippocampal CA1 injections of L-arginine increased morphine-induced conditioned place preference in male rats, and this effect was blocked by L-NAME; L-arginine or L-NAME itself did not induce conditioned place preference [38]. NO in the rat hippocampal CA1 area may be involved in the morphine-induced conditioned place preference. There have been findings suggesting that an increase in the NO levels in the central amygdala [39], the nucleus accumbens [40,41], and ventral tegmental area [42] may have an effect on the acquisition and expression of morphine-induced conditioned place preference in rats.

In mice, treatment with moderate and high doses (50 and 100 $mg \cdot kg^{-1}$, i.p.) of 7-NI produced conditioned place aversion, and the lowest dose used (25 $mg \cdot kg^{-1}$) blocked morphine-induced conditioned place preference; 7-NI did not affect the spontaneous locomotor activity or hyperactivity induced by morphine, suggesting that NO produced by nNOS is involved in the rewarding properties of morphine, but not in its motor effects [43]. Cyclosporin A (5 and 10 $mg \cdot kg^{-1}$, i.p.) and L-NAME neither induced conditioned place preference nor conditioned place aversion in mice, while cyclosporin A at a high dose (20 $mg \cdot kg^{-1}$) induced conditioned place aversion; both cyclosporin and L-NAME, in combination with morphine during conditioning, suppressed the acquisition of morphine-induced place preference, and aminoguanidine failed to show this inhibitory effect

[44]. Decreasing NO production through inhibiting constitutive, but not inducible, NOS may be a mechanism through which cyclosporin A attenuates morphine-induced place preference.

Convulsion

Acute subcutaneous administration of low doses (0.5–3 $mg \cdot kg^{-1}$, s.c.) of morphine increased the threshold of seizures induced by pentylenetetrazole in mice, whereas high doses (15–60 $mg \cdot kg^{-1}$) of morphine had proconvulsant effects; L-NA or L-NAME inhibited both the anticonvulsant and proconvulsant effects of morphine, and L-arginine potentiated both effects, suggesting the involvement of the L-arginine/NO pathway in the modulation of seizure threshold by morphine [45]. There was evidence suggesting that melatonin enhanced both the anti- and proconvulsant effects of morphine via a mechanism that may involve the NO pathway [46]. A prolonged period of intermittent foot-shock stress, which induced opioid-mediated analgesia, had protective effects against seizures induced by pentylenetetrazole and electroconvulsive shock, as did morphine; L-NAME, but not aminoguanidine, blocked the stress-induced anticonvulsant effects [47,48]. NO synthesis through constitutive but not inducible NOS appears to be involved in endogenous opioid-dependent stress-induced anticonvulsant effects.

Thermoregulation

The intracerebroventricular administration of selective μ opioid-receptor agonists produced hyperthermia, whereas κ-receptor agonists produced hypothermia [49,50]. NO has an antipyretic action [51] and is involved in hypothermia [52], while in contrast, other reports provide evidence for NO participation in the development of febrile responses [53,54].

The intraperitoneal administration of L-NA blocked morphine (15–105 $mg \cdot kg^{-1}$, i.p.)-induced hyperthermia in rats and this effect was reversed by L-arginine [55]. Benamar et al. [56] reported that morphine (4 and 15 $mg \cdot kg^{-1}$, s.c.) produced hyperthermia in rats, and the subcutaneous or intracerebroventricular administration of L-NAME suppressed the effect of morphine at the high dose but did not alter the effect of the low dose; L-NAME per se had no influence on body temperature. Either central or peripheral NO synthesis may be required for the production of hyperthermia induced by 15 $mg \cdot kg^{-1}$ of morphine; however, NO synthesis does not seem to be involved in the hyperthermic process induced by 4 $mg \cdot kg^{-1}$of morphine. Benamar et al. [57] also found that hyperthermia induced by morphine was blocked by 7-NI but was unaffected by the eNOS inhibitor N^5-(-iminoethyl)-L-ornithine (L-NIO) or aminoguanidine. It is possible that nNOS may be involved in morphine-induced hyperthermia. On the other hand, intraperitoneally administered morphine produced a hypothermic effect in mice, which was also elicited by intraperitoneal L-NAME or ketamine, and morphine-induced hypothermia was enhanced by L-NAME and ketamine, suggesting a possible role of the NMDA-NO pathway in the thermoregulatory effect of morphine [58].

There have been controversial findings as to the pyretic and antipyretic effects of NO and also concerning the thermoregulatory actions of morphine. There are, as yet, no comparative studies to determine whether differences in experimental conditions, such as drug doses, drug administration routes, and animal species, may be responsible for the differences in findings. It may be intriguing to draw attention to the interactions between thermoregulation and mitochondrial respiration that are regulated by NO and morphine.

Mitochondrial respiration

In cultured human glioma cells, morphine-induced NO release through the mediation of naloxone-sensitive receptors decreased the mitochondrial membrane potential [59], as one might expect, based on the rapid inhibition of the respiratory chain by NO [60]. Morphine was suggested to protect Purkinje cells against cell death under simulated ischemia/reperfusion conditions [61]; inhibition of the respiratory chain by NO may induce activation of glycolysis [62] and be beneficial for neurons exposed to ischemia [63].

Penile erection

The stimulation of dopaminergic receptors by N-n-propyl-norapomorphine produced recurrent episodes of penile erection in rats; this stimulant effect was prevented by morphine, as well as by haloperidol, and was potentiated by naloxone, suggesting that dopaminergic receptor stimulation appears to cause the release of opioid peptides, damping the sexual stimulant effect [64]. Oxytocin-induced penile erection and yawning in rats were prevented by atropine and morphine [65]. Stimulation of opioid receptors reduced penile erectile responses and seminal emission in rats [66]. Morphine prevents apomorphine (dopaminergic agonist)- and oxytocin-induced penile erection and yawning, possibly by inhibiting the activity of oxytocinergic neurons through μ-type receptors in the paraventricular nucleus of the rat hypothalamus [67]. Melis et al [68] reported that NMDA injected into the paraventricular nucleus of the rat hypothalamus induced penile erection and yawning and increased NO_2 and NO_3 (NOx), and that morphine, but not the κ opioid-receptor agonist

U-69,593, prevented the NMDA-induced increase in NOx concentrations, with a concomitant decrease in the number of penile erection and yawning episodes; the effect of morphine was antagonized by treatment with naloxone. Morphine appears to prevent the NMDA-induced increase in paraventricular NO production, penile erection, and yawning by inhibiting NOS activity through the stimulation of opioid receptors of the μ-subtype (Fig. 1). Melis et al. [69] also found that either subcutaneous apomorphine or intracerebroventricular oxytocin increased basal NOx concentrations in the paraventricular dialysate, and increased penile erection and yawning; these effects were prevented by the intraperitoneal treatment of male rats with morphine. Together with previous findings, these observations suggest that morphine acts through μ-receptors in the paraventricular nucleus to prevent apomorphine- and oxytocin-induced penile erection and yawning, and that the effect of morphine is mediated by the decreased activity of NO. Male rats showed penile erectile episodes when put in the presence of an inaccessible receptive female; these episodes occurred concomitantly with an increase in NO production in the paraventricular nucleus of the hypothalamus; morphine, but not U-69,593, given unilaterally into this hypothalamic nucleus, prevented NOx increases and noncontact erections, suggesting that morphine acts through μ-receptors in the paraventricular nucleus to impair noncontact erections and copulation [70]. Succu et al. [71] found that muscimol and morphine given into the paraventricular nucleus reduced penile erection induced by the hexarelin analog peptide EP 80661 injected into this hypothalamic nucleus, in association with a decrease of the NOx increase; these effects of muscimol and morphine were prevented by bicuculline and naloxone, respectively. The activation of γ-aminobutyric acid $(GABA)_A$ receptors and opioid receptors in the paraventricular nucleus appears to impair the penile erection induced by EP 80661 by reducing the increase in NO activity. Penile erection and concomitant increases in the concentrations of glutamic acid and NOx in the paraventricular dialysate induced by the cannabinoid CB1 receptor antagonist SR 141716A were reduced by morphine given into the paraventricular nucleus [72]. The activation of opioid receptors may impair the penile erection induced by SR 141716A by reducing the increases in glutamic acid and NO activity that occur in the hypothalamic nucleus. As reported so far, it appears that increased availability of NO in the paraventricular nucleus, as well as dopaminergic receptor activation, contributes to penile erection, and the activations of μ-opioid and $GABA_A$ receptors are involved in the impairment of penile erectile function.

It is widely known that penile erection in experimental animals and in men is mediated through NO released from parasympathetic nitrergic nerves, which results in the relaxation of cavernosal smooth muscle and an increase in intracavernous pressure [2,17,73]. Although the peripheral mechanisms of the erectile action of apomorphine, known as an aphrodisiac, have not been determined, Melis et al. [69] have suggested its central mechanisms of action. However, how the increased NO production induced by this and other aphrodisiacs in the paraventricular nucleus of the hypothalamus in rats participates in autonomic nitrergic nerve activation remains unidentified.

Regulation of secretion

L-NAME potentiated acute morphine-induced prolactin secretion and attenuated the subsequent tolerance to morphine in rats, whereas immobilization stress-induced prolactin secretion was inhibited by L-NAME, as was the subsequent tolerance to morphine [74]. Morphine subcutaneously injected to rat pups elicited increases in both adrenocorticotropic hormone (ACTH) and corticosterone secretion, these responses increasing with advancing postnatal age; naloxone, when concomitantly administered with morphine, was unable to block the morphine-induced responses, but in contrast, they were blocked by pretreatment with naloxone or L-NAME [75]. Endogenous NO is suggested to be one of the major factors in the response of the pituitary-adrenocortical axis to morphine.

Intracerebroventricular administration of the μ-opioid agonist D-Ala2, N-Me-Phe4, Gly-ol5 (DAMGO) to rats increased endogenous NO production by splenocytes stimulated with toxic shock syndrome toxin, and the effect was blocked by N-methylnaltrexone; in contrast, administration of the κ-agonist U69,593 and the δ-agonist [(D)-Pen(2,5)]enkephalin (DPDPE) had no effect on the production of NO; in vitro application of DAMGO, DPDPE, or U69,593 to splenocyte cultures did not alter the production of NO [76]. The μ-opioid receptor within the CNS appears to be involved in the regulation of macrophage NO production in splenocytes

Cardiovascular system

Vascular endothelium and smooth muscle

Stefano et al. [77] have provided evidence suggesting that morphine induces the production of NO in human arterial and rat microvascular endothelial cells, a process that is sensitive to naloxone antagonism and NOS inhibition and in which a μ3-opioid receptor subtype is coupled to NO release and vasodilatation (Fig. 2). The expression of μ-type opioid receptors was

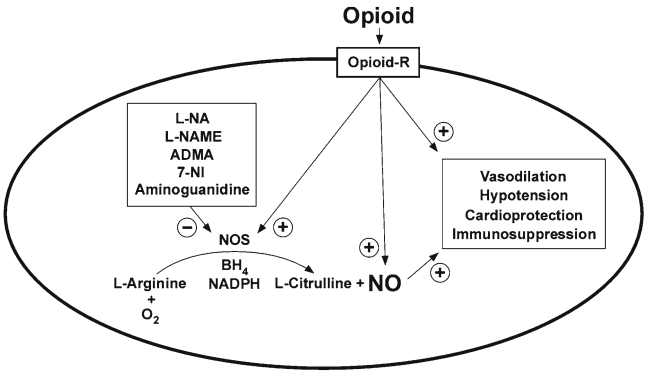

Fig. 2. Possible mechanisms of peripheral action of opioids through NO bioavailability on vasodilatation, hypotension, cardioprotection, and immunosuppression. (+), Stimulation; (–), inhibition. Endothelial NOS (eNOS) is involved in vasodilatation and hypotension,; constitutive NOS and inducible NOS (iNOS) are involved in cardioprotection and immunosuppression

suggested to be upregulated by proinflammatory cytokines in human vascular and cardiac endothelia [78]. In cultured human arterial endothelial cells, both morphine and the cannabinoid type 1 receptor agonist anandamide stimulated an increase in intracellular Ca^{2+} ($[Ca^{2+}]i$), which was blocked by their respective receptor antagonists, naloxone and SR171416A; an increase in $[Ca^{2+}]i$ preceded the stimulation of NO production [79]. Endothelial $[Ca^{2+}]i$ levels seem to regulate cNOS activity. In saphenous vein segments from patients with type 2 diabetes, treatment with morphine resulted in a lower peak and shorter duration of NO release from the endothelium compared to findings in saphenous vein segments from nondiabetic subjects, and μ-opioid receptor expression was diminished in the diabetic tissue [80]. Lactoferrin (LF) is a multifunctional protein that is found in milk and other biological fluids, and in neutrophils [81]; this compound produces analgesia via a μ-opioid receptor-mediated response in the spinal cord [82]. In anesthetized rats, intravenous bovine LF (BLF) decreased mean blood pressure but did not affect heart rate, while morphine decreased both mean blood pressure and heart rate; the hypotensive effect of BLF was abolished by L-NAME and attenuated by blood-brain barrier-permeable naloxone hydrochloride, but not by impermeable naloxone methiodide. Therefore, it was concluded that BLF caused hypotension possibly via an endothelium-dependent, NO-mediated vasodilatation and that the induced hypotension may also be mediated by the central opioidergic system [81] (Fig. 2).

From their findings that morphine and anandamide stimulated the release of gonadotropin-releasing hormone from median eminence fragments and that the NO release stimulated by these agents appeared to originate from the vascular endothelium, Prevot et al. [83] concluded that endothelial cells of the median eminence may be involved in the release of the neurohormone. Selective rises in rat lumbar dorsal cord blood flow were generated by ipsilateral, "nociceptive" low-frequency stimulation of sciatic afferents, and inhibitors of NOS prevented rises in flow; during NOS blockade or morphine administration, there were acute declines in the blood flow confined to low-frequency stimulation periods, and this effect was prevented by an opioid receptor antagonist. In addition, Zochodne et al. [84] reported that dorsal spinal cord blood vessels were labeled with antibodies against nNOS and met-enkephalin. These authors concluded that local NO and opioids, probably from interneurons, appeared to have competitive actions on dorsal horn microvessels once interneurons were activated during a nociceptive barrage, and that collateral innervation of blood vessels may explain this property.

There is evidence for μ- and δ-opioid receptor-independent or endothelium-independent vasodilatation induced by morphine. In anesthetized cats, combined administration of naloxone and L-NAME reduced the cerebral and spinal vascular responsiveness to hypercapnia, but neither μ- nor δ-opioid receptor blockade along with simultaneous NO blockade were able to decrease CO_2 responsiveness [85]. It was suggested that there is a previously unknown interaction between the NO system and the endogenous opioid system in the cerebrovascular bed during hypercapnic stimulation, with the phenomenon not being mediated by μ- or δ-opioid receptors. The involvement of NO in the hypercapnia-induced cerebral vasodilatation is still controversial [86]. In isolated rat small mesenteric arteries, morphine-induced relaxation was endothelium-independent and was not inhibited by L-NAME and indomethacin; the response to morphine was inhibited by naloxone and abolished when the arteries were exposed to Ca^{2+}-free media [87]. Relaxation induced by morphine, meperidine, fentanyl, and remifentanil in human radial artery segments was also independent of the endothelium [88].

Namiranian et al. [89] reported that acetylcholine (ACh)-induced vasodilatation, but not SNP-induced vasodilatation, in isolated perfused mesenteric artery was impaired in bile duct-ligated rats, and chronic treatment with L-NAME or naltrexone partially restored the response to ACh, suggesting that the impaired ACh-induced vasodilatation in cholestatic rats was due to a defect in endothelial function. These authors speculated that an increased level of endogenous opioids in bile duct-ligated rats chronically increased $[Ca^{2+}]i$ [90]; thus, ACh could no longer increase the $[Ca^{2+}]i$ level to stimulate cNOS to release NO.

Heart

Jiang et al. [91] found that infarct size in a heart subjected to coronary occlusion/reperfusion was reduced by morphine in wild-type mice, and this cardioprotective effect was abolished by S-methylthiourea sulfate and was absent in *iNOS*-gene-knockout mice; an increase in myocardial iNOS expression was observed after morphine administration. These authors suggested an obligatory role for iNOS in mediating morphine-induced delayed cardioprotection. The cardioprotective effect 24 h after morphine administration was abolished by the cyclooxygenase (COX)-2 inhibitor NS-398, and enhancement of myocardial COX-2 expression, together with upregulation of the myocardial contents of prostaglandin (PG) E_2 and 6-keto-$PGF_{1\alpha}$, was observed 24 h after morphine preconditioning; the iNOS inhibitor S-methylisothiourea and targeted abrogation of the *iNOS* gene blocked the upregulation of PGE_2 and 6-keto-$PGF_{1\alpha}$, but did not inhibit the enhancement of COX-2 expression [92]. COX-2 appears to mediate morphine-induced delayed cardioprotection via an iNOS-dependent pathway (Fig. 2), iNOS modulates COX-2 activity and iNOS-derived NO drives prostanoid synthesis by COX-2 in the rabbit heart during ischemic preconditioning [93]. In mice preconditioned with coronary occlusion, COX-2 activity required upregulated iNOS and iNOS-derived NO [94].

Brief ischemic preconditioning of the myocardium downregulates the excitatory state of the cells via cNOS-derived NO, thus protecting myocardial cells from the next insult; opioid actions may be incorporated into the protection scenario, possibly through NO release [95]. Postconditioning elicited by repeated cycles of ischemia and reperfusion reduced infarct size in open-chest rats, and this effect could be reversed by naloxone, the δ-opioid receptor antagonist naltrindole, and the mitochondrial permeability transition pore (mPTP) opener atractyloside; in isolated hearts, morphine as well as postconditioning reduced infarct size, and L-NAME and ODQ blocked the action of morphine; morphine produced NO in cardiomyocytes by activating δ-opioid receptors [96]. Postconditioning appears to protect the heart from reperfusion injury by targeting the mPTP through the activation of δ-opioid receptors that mediate NO release.

Digestive system

Morphine-induced constipation in mice was suggested to depend on actions on the CNS, based on the findings that N-methyl morphine, a quaternary derivative, inhibited intestinal transit only when administered intracerebroventricularly; L-arginine given intraperitoneally, but not when given intracerebroventricularly, reversed the constipation. L-arginine appears to modulate opioid-induced constipation possibly through increasing the amount of NO released from nitrergic nerves in the gut [97]. It may represent a useful agent for the treatment of undesirable constipation. In the duodenum of anesthetized rats, atropine, muscarinic M_1- and M_3-receptor antagonists, or L-NA augmented the spontaneous contractile activity, and this effect was abolished by ganglionic blockade or by morphine [98]. The excitatory motor activity in the rat duodenum seems to be modulated by nitrergic or opioidergic inhibitory mechanisms. In the circular muscle of guinea pig ileal segments treated with atropine, morphine caused tonic contraction, which was reversed by naloxone; tetrodotoxin also caused contraction and morphine lost its effect in the presence of tetrodotoxin; L-NA elicited tonic contraction and prevented the excitatory effect of tetrodotoxin or morphine [99]. It appears that NO-releasing myenteric neurons exert a tonic inhibitory influence on the circular muscle of the guinea pig ileum and that morphine and tetrodotoxin contract the circular muscle by reducing the amount of NO released. Tonic nitrergic inhibitory innervation has been widely recognized in the gastrointestinal tracts of various mammals [100]. In isolated mouse ileal circular muscle, morphine caused tonic contraction that was inhibited by naloxone and the μ-opioid receptor antagonist cyprodime and abolished by either tetrodotoxin or L-NA [101]. Tucci et al. [102] have provided evidence that papaverine counteracts the morphine-induced inhibition of gastrointestinal transit in mice and that papaverine exerts its action through a NOS-mediated mechanism, and this mechanism does not operate when capsaicin-sensitive afferent neurons are ablated.

Based on both in vivo and in vitro studies of the gut, morphine-induced constipation may be ascribed to inhibition of the tonic activation of nitrergic nerves that are involved in gastrointestinal smooth muscle relaxation and also in sphincteral opening. Based on these observations, it would be very interesting to speculate that the impairment induced by centrally acting morphine on parasympathetic nitrergic nerve function shares mechanisms underlying the constipation associated with decreased gastrointestinal and sphincter muscle relaxation and penile erectile dysfunction (see "Penile erection" section under heading "CNS" above).

Morphine decreased the mucosal lesions induced by ethanol or acidified aspirin in rats, and when the animals were pretreated with L-NA, the mucosal lesions were exacerbated and the gastroprotective action of morphine was decreased; when L-NA was given simultaneously with indomethacin, the protective effect of morphine was completely inhibited [103]. Endogenous NO is likely to be involved in the gastro-

protective action of morphine, the protective action of NO being independent of mucosal prostaglandins. Stefano et al. [104] noted that morphine stimulated constitutive NO release in the mouse stomach, small intestine, and large intestine in a naloxone- and L-NAME-sensitive manner and surmised that morphine acts as a hormone to limit gut activity via μ3 receptors coupled to NO release; μ3 receptors are found in the gut; endogenous morphine is not found in the gut but it is found in blood.

According to Abdollahi and Safarhamidi [105], intraperitoneal injection of morphine in rats reduced the salivary flow rate in the submandibular gland and reduced total protein and Ca^{2+} concentrations in saliva; L-NAME reduced the salivary flow rate and decreased the total protein and Ca^{2+} levels of saliva, L-arginine increased the protein and Ca^{2+} concentrations, and L-NAME potentiated, and L-arginine prevented, the inhibitory effects of morphine. These authors suggested that morphine inhibits salivary gland function and that NO plays a positive role in this system.

Kim and Lemasters [106] noted that in cultured rat hepatocytes, morphine improved cell viability after anoxia/reperfusion and increased NO generation, which was attenuated by ATP-sensitive K^+ channel blockers; however, treatment with opioid receptor antagonists did not reverse the cytoprotection conferred by morphine, indicating that morphine prevents anoxia/reperfusion injury to hepatocytes and that the protective mechanisms may be associated with K^+ channels and NO, but are independent of opioid-mediated signaling.

Cholestasis induced chemically in mice increased the threshold to generate clonic seizures induced by pentylentetrazole, an effect that was inhibited by naltrexone or L-NAME, suggesting that cholestasis leads to an increased resistance to seizure through an opioid/NO-mediated pathway [107].

Naloxone-induced withdrawal contracture in isolated guinea pig ileum exposed to morphine was attenuated by L-NAME or methylene blue; treatment with an NMDA-receptor antagonist reduced the amplitude of the naloxone-induced contracture, an effect that was reversed by co-administration of L-glutamate, confirming the involvement of the NO/NMDA pathway in morphine dependence [108]. After in vitro exposure to morphine, a selective μ-opioid receptor agonist, or a κ-opioid receptor agonist, the isolated guinea-pig ileum exhibited contracture in response to naloxone; pretreatment with L-NAME reduced the naloxone-induced contracture, and nitroglycerin increased the naloxone-induced response [109]. NO may have a role in the development of opioid withdrawal, and μ- or κ-opioid receptors are involved in this process.

Respiratory system

In awake dogs, L-NA perfused through the fourth ventricle reduced Pa_{CO_2}, and morphine elicited an increase in Pa_{CO_2} and a decrease in ventilatory drive; with L-NA pretreatment, but not posttreatment, the morphine-induced ventilatory depression was attenuated, and this inhibitory effect was reversed with the NO donor SNAP [110]. Endogenous NO, produced at a supraspinal site, appears to act as a ventilatory depressant, and NOS inhibitor treatment may be effective in suppressing morphine-induced ventilatory depression when given before morphine administration. Teppema et al. [111] reported that, in anesthetized cats, L-NA reduced ventilatory CO_2 sensitivity but decreased the mean apneic threshold. These authors [112] also noted that 7-NI reduced peripheral and central CO_2 sensitivities but decreased the mean apneic threshold, whereas morphine increased the apneic threshold and reduced CO_2 sensitivity; naloxone reversed the ventilatory effects of morphine, but not those induced by 7-NI. It appears that 7-NI and morphine have independent and partly opposing effects on the control of breathing in anesthetized cats and that the effects of 7-NI do not result from interaction with opioid receptors.

Immune system

Studies in experimental animals

White blood cells

Morphine induced the rounding and inactivation of spontaneously activated (mobile) ameboid monocytes and granulocytes within 20 min; these cells became rounded 24 h after morphine exposure, and the effects of morphine were blocked in the presence of L-NA; increases in NO concentration were observed 2 min after morphine stimulation, and this effect was impaired by L-NA and naloxone [113]. In awake mice, morphine was found to attenuate leukocyte rolling and sticking in both the arterial and venular side of the microcirculation, and this attenuation was reversed by naloxone and L-NA, but not by aminoguanidine, indicating that morphine interferes with leukocyte-endothelial cell interactions via the stimulation of NO production by cNOS [114].

In mice, cytostasis and NO_2 production in macrophages, activated by exposure to L1210 leukemic cells, were enhanced by morphine treatment immediately after drug injection, while, in contrast, morphine induced a strong inhibition of both cytostasis and NO_2 production 24 h after treatment; NO_2 production was inhibited by dexamethasone [115]. In vivo administration of morphine appears to induce a modulation of NO

biosynthesis by peritoneal macrophages. Morphine increased macrophage-derived NO and decreased lymphocyte proliferation in concanavalin-A-stimulated rat splenocyte cultures, and the addition of the hemoglobin or guanylyl cyclase inhibitors, methylene blue and LY 83583, attenuated the suppressive effect of morphine on proliferation [116]. These results suggest that the in vivo administration of morphine increases the synthesis and release of NO from macrophages and that the activation of soluble guanylyl cyclase by NO accounts for the morphine-induced suppression of lymphocyte proliferation.

Morphine enhanced apoptosis in cultured colonic cells, together with the stimulation of NO generation by these cells, and NOS inhibitors attenuated the proapoptotic effect of morphine; mice receiving morphine showed a breach in the host defense barrier, as well as injury to peritoneal macrophages. Although NOS inhibitors prevented morphine-induced intestinal ulcer formation, this treatment provided only partial protection against the breach in the host defense barrier and peritoneal macrophage injury [117]. Morphine-induced intestinal injury may be mediated through NO generation; however, degradation of the host defense barrier is likely to correlate with macrophage injury, but not with intestinal injury. Morphine enhanced murine macrophage apoptosis, promoted NO production both under basal and LPS-stimulated states, increased macrophage mRNA expression of iNOS, and promoted the synthesis of Bax and p53 proteins by macrophages; L-NAME and L-NMMA attenuated the morphine-induced generation of NO [118]. It appears that morphine-induced macrophage apoptosis may be mediated through the generation of NO, and that morphine may activate the induction phase of the apoptotic pathway through p53 accumulation. Treatment of *Leishmania donovani*-infected mouse peritoneal macrophages with low concentrations of morphine endowed these cells with naloxone-sensitive leishmanicidal activity; aminoguanidine blocked the morphine-induced protective effect [119].

The use of heroin is associated with a high incidence of infectious disease. Subcutaneous heroin administration in rats resulted in a reduction in LPS-induced expression of iNOS mRNA in spleen, lung, and liver tissues and a decrease in the level of plasma nitrite/nitrate; these effects of heroin were blocked by naltrexone prior to the injection of heroin, suggesting that a reduction of NO production may be involved in the increased incidence of infectious diseases among heroin users [120].

Edema

Comert et al. [121] noted that the administration of morphine inhibited pulmonary edema and reduced iNOS immunohistochemical staining induced by α-naphthylthiourea in rats; the protective effect of morphine against pulmonary edema was prevented by the peripheral opioid receptor antagonist naloxone methiodide. The preventive effect of morphine against the chemically induced pulmonary inflammatory reaction, which was possibly associated with increased production of NO, appears to be mediated via peripheral opioid receptors. The intrathecal administration of morphine inhibited carrageenan-induced edema in the rat paw, and the co-injection of naloxone and a subeffective dose of L-NA or ODQ with morphine prevented its antiedematogenic effect, supporting the idea that morphine acts on opioid receptors at the spinal level to inhibit inflammatory edema and that the NO/cyclic GMP pathway seems to be an important mediator of this effect [122].

Glial cells

Morphine protected primary rat neonatal astrocytes from apoptosis mediated by sodium nitroprusside, and 3-morpholinosydnonimine, a donor of peroxynitrite, inhibited the nuclear condensation and fragmentation of SIN-1-treated cells; the effects of morphine on SIN-1-induced cytotoxicity were prevented by pretreatment with a G_i protein inhibitor or phosphatidylinositol 3-kinase (PI_3 kinase) inhibitors [123]. Morphine may protect primary rat astrocytes from the apoptosis induced by NO species via signaling cascades that involve G protein and PI_3 kinase. Duan et al. [124] provided evidence suggesting that, in the leech, the first step in the repair process in the CNS was the rapid migration of microglia toward the axonal injury, that was controlled in part by NO. According to Yahyavi-Firouz-Abadi et al. [125] morphine decreased the injury-induced microglial accumulation in the leech, and L-NAME and naloxone reversed the inhibitory effect of morphine. It is hypothesized that injury-induced NO production may serve as a stop signal for migrating microglia and thereby disrupt the injury-induced NO gradient along the leech nerve cord.

Studies of human materials

NO was released from human saphenous vein endothelial cells exposed for 2 h or longer to LPS and interferon (IFN)-γ, consistent with iNOS activation; preincubation with morphine or anandamide before the addition of LPS + IFN-γ blocked the iNOS activity, and exposure to the NO donor SNAP blocked the iNOS induction, whereas the administration of NOS inhibitors, before morphine or anandamide exposure, restored the LPS + IFN-γ induction of iNOS [126]. After short-term exposure of human saphenous vein or internal thoracic artery endothelium to morphine or anandamide, mono-

cyte adherence was diminished, whereas it was enhanced with exposure to the human immunodeficiency virus envelope protein gp120, suggesting that, in individuals abusing opioids and/or cannabinoids, the tissue viral load may be higher, and acquired immunodeficiency syndrome may progress more rapidly in such individuals because monocyte adherence and mobility is increased [127]. Unlike exposure to morphine, the exposure of these human vessels to a δ_2 ligand-binding opioid peptide, [^{3}H] Ala2-met5 enkephalinamide, enhanced granulocyte adherence, did not stimulate NO release, and prevented the morphine-stimulated release of NO [128]. Opioid peptides and opioid alkaloids may regulate endothelial function in an antagonistic manner. Preincubation of human atrial fragments with morphine, anandamide, or estrogen prior to the addition of LPS + IFN-γ blocked iNOS expression; SNAP also blocked iNOS induction, and L-NAME restored the LPS + IFN-γ induction of iNOS, suggesting a direct regulatory link at the transcriptional level between cNOS and iNOS in human atrial tissue [129]. Morphine is capable of inducing both cNOS- and iNOS-coupled NO release that regulates the activation state of human macrophages [130]. Morphine, via NO, was suggested to have the potential to diminish the expression of adhesion molecules and in so doing, to attenuate the inflammatory process between human immunocytes and the endothelial surface [131]. Morphine protects human neuroblastoma cells against damage caused by peroxynitrite [132]. Morphine inhibited LPS-induced nuclear factor (NF)-κB nuclear binding in human blood neutrophils and monocytes in a naloxone-sensitive manner, and similar effects were achieved with SNAP and the antioxidant N-acetyl-cysteine; L-NAME and L-NA abolished the morphine-induced attenuation of NF-κB nuclear binding [133]. Morphine seems to cause immunosuppression, at least in part via the NO-stimulated depression of NF-κB nuclear binding. Welters et al. [134] have also provided evidence that neutrophil functions are inhibited by morphine-stimulated NO release, mediated by the μ-opioid receptor subtype found on immunocytes. Fentanyl had no immunosuppressive effects.

Human white blood cells release morphine into the environment to regulate themselves and other cells [135]. Incubation of human blood with morphine and LPS decreased activator protein-1 nuclear content in a naloxone- or NOS inhibitor-sensitive manner; CD14 expression was reduced after morphine treatment, and the effects were antagonized by NOS inhibitors and naloxone [136]. Morphine appears to inhibit activator protein-1 activation by a μ-opioid receptor pathway coupled to NO. Activation of phagocytes by LPS depends on the expression of CD14. The decrease in CD14 expression caused by morphine may play a role in the inhibition of activator protein-1 activation following the treatment of phagocytes with LPS.

As investigated so far, morphine shows immunosuppressive effects via NO production in human tissues (Fig. 2), as has been found in experimental animals in vivo and animal tissues in vitro.

Other studies

Effects on eyes

Drago et al. [137] showed that acute intraocular injection of morphine in rabbits decreased intraocular pressure, and this effect was prevented by the conjunctival instillation of naloxone. In addition, patients with chronic open-angle glaucoma showed a decrease in intraocular pressure after the conjunctival instillation of morphine; intraocular pressure was lower in patients addicted to morphine or heroin than in control subjects, and the instillation of naloxone increased intraocular pressure in addicted patients. These authors concluded that intraocular opioid receptors are involved in the regulation of intraocular pressure in animals and humans. In conscious, dark-adapted white rabbits, instillation of morphine decreased intraocular pressure and pupil diameter in a naloxone-dependent manner; pretreatment with either L-NAME or reduced L-glutathione, a μ3-opioid receptor antagonist, inhibited these morphine effects [138,139]. It appears that biochemical mechanisms related to NO release are involved in the effects of morphine on the eye, and μ3-opioid receptors may mediate morphine-induced miosis and reduction in intraocular pressure. Dortch-Carnes and Russell [140] also noted that morphine caused an increase in the levels of NO in the aqueous humor in dark-adapted rabbits, and the morphine-induced increase in NO levels was completely inhibited in the presence of naloxone, L-NAME, or reduced glutathione, suggesting that the morphine-stimulated changes in ocular hydrodynamics and iris function are due to an increased release of NO in aqueous humor that is mediated by the activation of μ3-opioid receptors.

Miscellaneous findings

Budziszewska et al. [141] found that single or repeated administrations of morphine increased plasma corticosterone in mice, and repeated, but not single, administration of L-NAME elevated the hormone level; pretreatment with L-NAME enhanced the stimulatory effects of morphine administration. On the other hand, repeated morphine injections decreased the plasma testosterone concentration, and pretreatment with L-NAME did not affect the hormone level in the morphine-treated mice. It appears that inhibition of NO

synthesis enhances the stimulatory effect of morphine on corticosterone secretion, but does not influence the inhibitory effect of morphine on the plasma testosterone level.

Pharmacological analyses performed by Cadet et al. [142] have provided validating evidence of functional μ3-like opioid receptor/NO-coupled signaling within primary cultures of undifferentiated human multilineage progenitor cells via the morphine-evoked release of NO. These authors suggested the primordial regulatory role of μ3-like opioid receptor/NO signaling in embryogenesis.

Morphine-coupled NO release is neuroprotective via inhibitory effects on the expression of β-secretase, a key protease involved in the maturation of β-amyloid protein in the CNS of Alzheimer patients [32]. The inhibition of β- and γ-secretase decreases endothelial cell proliferation, opposing the sprouting of microvessel outgrowth [143]. Stefano et al. [144] have hypothesized that one of the pathways involved in the antitumoral potential of morphine may be via the NO-dependent modulation of secretase activity; that is, decreased angiogenesis.

Conclusions

This article summarizes useful information concerning the interactions between morphine/other opioids and NO in the central and peripheral nervous systems and in the cardiovascular, digestive, respiratory, and immune systems. In the section on the CNS, up-to-date findings on learning, memory, brain mitochondrial respiration, convulsion, thermoregulation, and penile erection are included. The morphine-induced impairment of memory formation is prevented by NO, the increased production of NO in the brain is involved in morphine-induced conditioned place preference, and the erectile dysfunction induced by morphine is associated with decreased availability of brain NO. Morphine liberates NO from brain tissues, spinal cord, and vascular endothelial cells. Morphine-induced NO release inhibits the respiratory chain in brain-cell mitochondria. The LPS-stimulated iNOS expression in macrophages is either decreased or increased by morphine in experimental animals, whereas morphine, via NO, has the potential to cause immunosuppression in human tissues. Although the reasons for the controversial results on morphine-NO interactions in experimental animals remain to be elucidated, the information included in the present review could contribute to the construction of advanced strategies for therapy with morphine, with the goals of minimizing side effects. However, the necessary information about morphine-NO interactions in healthy humans and patients is still insufficient and rather inconsistent.

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