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Kirk Mantione, Yannick Goumon, Tobias Esch, George Stefano

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Morphine 6β glucuronide: Fortuitous morphine metabolite or preferred peripheral regulatory opiate?

Kirk J. Mantione[1], Yannick Goumon[2], Tobias Esch[3], George B. Stefano[1]

[1] Neuroscience Research Institute, State University of New York College at Old Westbury, Old Westbury, NY, U.S.A.
[2] Inserm, 575 "Physiopathology of the Nervous System", Strasbourg, France
[3] Charité – University Medicine Berlin, Institute for General Practice and Family Medicine, Berlin, Germany

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Summary

Morphine-6β-glucuronide (M6G), a metabolite of morphine that the brain can produce, is an opiate agonist that appears to have a greater analgesic potency than morphine. M6G has a 1-octanol/water partition coefficient 187 times lower than that of morphine and M6G has a blood brain barrier permeability 57 times lower than morphine. The brain uptake rate however is only 32 times lower, suggesting that an active transport mechanism might be present. Furthermore, evidence for a distinct receptor for M6G also appears to be emerging. Real time polymerase chain reactions allowed for the discovery of single nucleotide polymorphisms (SNP's) in the human mu opioid receptor gene. The most common SNP is a substitution at base118 where A is replaced with G (A118G). This SNP has a decreased potency for M6G in individuals possessing it whereas the potency of morphine is unaffected by this SNP. The possibility that a peripheral opiate signaling system, using M6G and its distinct receptor, exists seems plausible. Taken together, if a distinct M6G signaling mechanism does exist, the fact that morphine can be converted into a more water soluble compound that might be more potent would not be an accident.

key words: **morphine • morphine 6 glucuronide • nitric oxide • immune • nervous • vascular • gut • mu opiate receptor**

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Author's address: George B. Stefano, Neuroscience Research Institute, State University of New York College at Old Westbury, Old Westbury, NY, U.S.A. email: gstefano@sunynri.org

BACKGROUND

Morphine-6β-glucuronide (M6G), a metabolite of morphine, is an opiate agonist that has been demonstrated to have a greater analgesic potency than morphine [1,2]. Morphine and M6G stimulate constitutive nitric oxide (NO) synthase-derived NO release at comparable concentrations as in invertebrate ganglia [3]. Morphine can be converted to M6G and morphine-3-glucuronide (M3G) by the human UDP-glucuronosyltransferase 2B family (UGT2B) in the liver and brain [4–6]. At physiologic concentrations of morphine (endogenous morphine), the human brain is capable of converting morphine to M6G [5]. In humans, about 55% of exogenously administered morphine can be converted to M3G, and between 5–15% can be converted to M6G principally in the liver [7]. However, the brain and kidneys may also play a role in its conversion. UGT's in the liver act as detoxification enzymes and can act on various other compounds to facilitate their excretion by the kidneys.

Recently, Okura et al. [8] have suggested that the increased analgesic potency of M6G might not be due to increased efficacy, but rather to the higher concentrations of M6G at the receptor sites in the central nervous system. This hypothesis is in agreement with our observations, concerning mu opiate receptor efficacy and NO release [3,9].

The greater analgesic efficacy, which has been noted [35,36], may be due to its solubility. M6G has a 1-octanol/water partition coefficient 187 times lower than that of morphine and it exhibits a blood brain barrier permeability 57 times lower than morphine [10]. The brain uptake rate, however, is only 32 times lower [10], suggesting that an active transport mechanism might be present.

In this regard, inhibition of P-glycoprotein in porcine brain capillary endothelial cells permits the transport of M6G [11]. In other experiments to elucidate the transporters involved with M6G at the blood brain barrier (BBB), Bourasset and co-workers [12] have demonstrated that P-glycoprotein does not mediate M6G's active transport at the BBB in mice, but digoxin and PSC833 decreased the uptake of M6G at the BBB. These researchers, as well as Skarke et al. [13], have also demonstrated that probenecid interacts with an M6G transporter, but not at the BBB. In addition, Tunblad et al. [14] have compared M6G's BBB transport and systemic elimination in the presence of probenecid. They concluded that there was a net efflux of M6G from the brain and that probenecid had no influence on the rate. However, the systemic elimination of M6G was decreased by 22% in the presence of probenecid. It was also noted that the half-life of M6G was longer in the brain than in blood [14]. These studies note that there are specific cellular processes that are involved with M6G processing, suggesting that it has its own signaling role that may either be the same as that of morphine or different (Figure 1).

A UNIQUE RECEPTOR?

The possibility of a distinct receptor for M6G was first proposed in 1995 by Rossi and colleagues [15]. Using an antisense probe targeting G-protein alpha subunit in mammals, they found that it blocked both heroin and M6G analgesia, but not that of morphine [16]. Morphine's and M6G's effects on analgesia, locomotion, feeding, respiration, and gut motility have all been compared [17–19]. Receptor binding studies and functional studies have also provided evidence for a selective M6G receptor [3,20]. Experiments with mice lacking exon 1 of MOR-1 revealed differences in sensitivity to morphine and M6G [21]. Mice lacking this exon were insensitive to morphine but not to M6G or heroin.

LOCOMOTOR ACTIVITY

In locomotor activity experiments with mice, there was a dose dependent development of tolerance to daily injections of M6G [22]. These investigators also found that there was a lack of cross-tolerance toward M6G after 1 day of morphine pretreatment, whereas cross-tolerance to M6G was observed after 7 days of exposure to morphine pretreatment. They concluded that the main part of the effect by M6G on locomotion was mediated through a specific M6G receptor. Locomotor stimulation in mice by M6G was compared to morphine in the presence of naltrexone or naloxonazine [17]. Morphine induction of activity was blocked by 0.5 mg/kg of naltrexone but M6G was only partially blocked. The μ_1 specific antagonist, naloxonazine, was able to block 75% of morphine induced activity, but only 50% of the M6G effect. These experiments provide further evidence for the possibility of two distinct receptors for these compounds.

FEEDING AND RESPIRATION

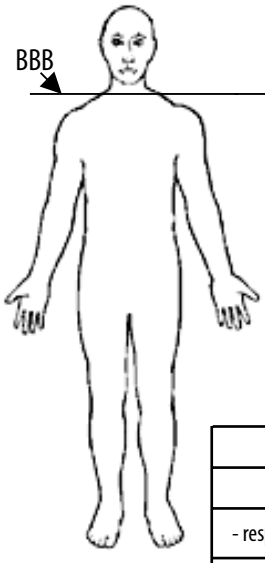
Studies investigating differences in morphine and M6G-induced feeding in rats also strongly suggest a novel M6G receptor. Silva and co-workers [23] present data that indicate that morphine and M6G-induced feeding are controlled through different G- protein α-subunits. Investigations with MOR-1 knockout mice lacking exon 2 revealed no difference in respiratory depression noted between morphine and M6G [24]. However, in human studies, morphine was more potent at affecting hypoxic ventilatory control than M6G [24], which again may be related to its solubility or its receptor binding specificity.

FUNCTIONAL BINDING STUDIES

Recent studies of opioids, opiates and their antagonists have revealed tissue-type- dependent differences in their interactions with receptors. Experiments have shown that peripherally acting antagonists may block opioid receptors in the gastrointestinal tract, but not those receptors found in the central nervous system [19]. These authors suggest that the μ receptor agonists that affect gut motility act in the periphery while μ receptors that affect analgesia are in the central nervous system.

Studies by our laboratory previously demonstrated that in murine macrophages the μ_3 receptor bind morphine, M6G, and other alkaloids [3]. The μ_3 receptor does not bind M3G and all opioid peptides tested [3]. Our study also noted that M6G had approximately a two-fold lower affinity for this receptor subtype than morphine, indicating a separate receptor for these two alkaloids, suggesting that M6G may function as a peripheral opiate hormone.

Nitric oxide signaling has been studied extensively in vascular, neural, and immune cells by our laboratory [25–29]. In



- Brain (central nervous system)
- converts low levels of morphine to M6G (active metabolite) and M3G (inactive)

- Peripheral systems
- Immunomodulation
- respiratory depression
- maintain vascular tone, cardioprotection
- Morphine to M6G and M3G in liver and kidneys (at higher levels than brain)
- M3G excreted via kidneys

Morphine promotes	M6G promotes
- analgesia	- analgesia (more potent)
- respiratory depression (more potent)	- respiratory depression
Down regulation of immune, vascular and nervous tissue via μ_3 opiate receptor coupled to NO	Down regulation of immune, vascular and nervous tissue via NO release

Figure 1. Morphine and M6G signaling.

Mytilus neural tissue, the general opiate -receptor antagonist naloxone failed to block M6G induced NO release [3], indicating that there may be a different receptor. CTOP, however, blocked both morphine and M6G induced NO release **[3]**. Other investigations by our laboratory have demonstrated that M6G as well as morphine can stimulate constitutive nitric oxide synthase in a variety of tissue types that contain the μ_3 opiate receptor [30]. Morphine-stimulated-NO release (derived from cNOS) produces NO at the nM level. This cNOS-derived NO has been shown to prevent the induction of inducible nitric oxide synthase [31]. Experiments by Lysle and Carrigan [32] used M6G to alter the expression of inducible nitric oxide synthase in rats with similar results. The reduction in iNOS expression and NO production was reversed using the opioid receptor antagonist, naltrexone. In light of these observations, it appears that the same NO signaling mechanism is responsible for the inhibition of iNOS by morphine and M6G.

Genetic Evidence of a Receptor

Following the first reports of genetic evidence for the M6G receptor [15], numerous experiments with knockout mice provided a way for exploring the possibility of various splice variants of the mu opioid receptor (as reviewed in [33]). Real time polymerase chain reactions allowed for the discovery of single nucleotide polymorphisms (SNP's) in the human mu opioid receptor gene. The most common SNP is a substitution at base118 where A is replaced with G (A118G). This SNP was first reported by Bond and co-workers [34]. This SNP predicts an amino acid change at position 40 of the protein where asparagine is replaced with an aspartatic residue. Early reports testing binding affinities for this mutated receptor demonstrated that it has a stronger affinity for beta-endorphin than for morphine [34]. Measurement of the degree of pupil constriction in humans revealed a decreased effect of M6G when compared to morphine [35]. Furthermore, Lotsch's group [36] also performed a small scale experiment on humans with this SNP and found a decreased potency of M6G in those individuals possessing it. Interestingly, the potency of morphine was unaffected by this SNP. Perhaps the receptor coded for by this mutant gene might have altered binding of molecules larger than morphine. Remembering that the A118G polymorphism is located within exon 1 [37], the results of Lotsch and others [35] contrast with the findings of Rossi and others as noted earlier [16]. Rossi's group, using knockout mice for exon 1 of the mu opioid receptor, show that the potency of M6G is not lost, but the potency of morphine is. In light of this, more work is needed to clarify these cross-species differences. The most recent experiment on the effects of the A118G SNP did not show any marked differences with respect to morphine and M6G when comparing binding affinity, sensitization and potency in human cells [38].

Conclusions

The possibility that a peripheral opiate signaling system, using M6G and its distinct receptor, exists seems plausible. Endogenous morphine and M6G have been found in mammals (man) and invertebrates (mussels) [39,40]. Opioid processes probably evolved over 500 million years ago and appear to be retained. Evidence for the conservative nature of the opioid signaling system has been provided by the high level of sequence similarity seen between man and mussel [41]. Several studies have shown that the level of en-

dogenous opiates in animals increases after stress [42–44], suggesting their role as a defense against physical and mental stressors. If this system does exist, the fact that morphine can be converted into a more water soluble compound that might be more potent would not be an accident. Therefore, given its lower level of BBB penetration it may serve as a peripheral morphinergic chemical messenger, i.e., hormonal, as suggested by μ_3 presence in various body organs, i.e., circulatory, immune, gut.

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