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INVITED NEL REVIEW

A Hormonal Role for Endogenous Opiate Alkaloids: Vascular Tissues

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Abstract The distribution of morphine-containing cells in the central nervous system, adrenal gland, and its presence in blood may serve to demonstrate that this signal molecule can act as a hormone besides its role in cell-to-cell signaling within the brain. This speculative review is the result of a literature evaluation with an emphasis on studies from our laboratory. Opioid peptides and opiate alkaloids have been found to influence cardiac and vascular function. They have also been reported to promote ischemic preconditioning protection in the heart. Given the presence of morphine and the novel μ_3 opiate receptor on vascular endothelial cells, including cardiac and vascular endothelial cells in the median eminence, it would appear that endogenous opiate alkaloids are involved in modulating cardiac function, possible at the hormonal level. This peripheral target tissue, via nitric oxide coupling to μ opiate receptors, may serve to down regulate the excitability of this tissue given the heart's high performance state as compared to that of the saphenous vein, a passive resistance conduit. With this in mind, morphine and other endogenous opiate alkaloids may function as a hormone.

Introduction

Adrenal Gland

Endogenous morphine has been identified in mammalian and invertebrate neural tissues by various techniques, including gas-chromatography mass spectrometry (GC/MS) [1–11]. Additionally, morphine has been found to exist in the vertebrate adrenal gland by way of immunocytochemistry and radioimmunoassay [1,12–19]. Recently, we also found morphine present in the rat adrenal gland and determined its identity by way of gas-chromatography mass spectrometry (GC/MS) [20].

We have extended these studies to include the demonstration of morphine in the rat adrenal medullary chromaffin PC-12 cell line by high performance liquid chromatography (HPLC) coupled with electrochemical detection and GC/MS [20]. We recently confirmed the presence of opiate alkaloids in this cell line using nano electrospray ionization double quadrupole orthogonal acceleration time of flight mass spectrometry (Q-TOF MS)[21]. Also, in this report, the major morphine metabolite, morphine 6-glucorinde (M6G) was identified. A single charge ion with a mass of 462.17 da is also present in the PC12 extract [21]. This value is identical to the calculated mass of M6G and is identical to the mass obtained from the analysis of authentic M6G [21].

We have recently reviewed the central nervous systems "morphinergic" system [22]. Within this context and that which is noted for the adrenal gland, morphine has the potential to be released into the circulatory system, thus, making it a hormone. Morphine is present in human and animal plasma, further suggesting hormonal action [23–27]. Thus, we are left with the question of what are the peripheral morphinergic targets. Based on the work of our laboratory, we surmise that these targets include, and are not limited to, immune and vascular tissues. In the past we have discussed the role of endogenous morphine in immune tissues [22,25,28–30]. In this review, we will examine studies suggesting a vascular role for this new hormone.

Discussion

We will define opioid peptide and opiate alkaloid by the strictest definition since an opiate alkaloid selective and opioid peptide insensitive binding site have been discovered on human immunocytes and vascular endothelial cells (Table 1), designated μ_3; [22, 28,31–33] and morphine has been demonstrated to be an endogenous signaling molecule [22,34].

Recent data supports the view that both opioid peptides and morphine exert important physiological functions in cardiac tissues. Naloxone has been shown to block the infarct-limiting effect of ischemic preconditioning [35,36], suggesting either opioid and/or opiate involvement. This finding also occurred in regard to repeated percutaneous transluminal balloon inflations [37]. Morphine administration to either rats or rabbits mimics the infarct-limiting effect of ischemic preconditioning [36,38], supporting a role for this signaling family in this phenomenon. Adding additional weight to opiate alkaloid involvement is our finding that human vascular tissues contain morphine and morphine-6-glucuronide [39].

In regard to δ-type opioid ligands, TAN-67 can mimic the protective effect found with ischemic preconditioning, limiting the infarct size [40,41] – a phenomenon blocked by the δ1-opioid receptor antagonist BNTX [41]. Other studies have also examined and supported a role for δ-opioid receptor mediated preconditioning [36,41–45]. Interestingly, publications demonstrating enkephalins cardioprotective actions are lacking. As suggested by [46], this may be due to their short-half life due to the presence of many types of degrading enzymes [47] and their susceptibility to such actions. However, given their presence in myocardial tissues (as noted above), and in various immunocytes, this protective action as occurring during some form of stimulation cannot be ruled out see [30,48]. This is noted by the fact that during a myocardial ischemic, Met5-enkephalin levels increased [49].

In another study, Liang and colleagues [50] found that morphine preconditioning occurred at 1 μM in cultured neonatal chick cardiomyocytes whereas, in another study, enkephalin preconditioning in the same cells occurred at 100 μM [46]. The authors could not provide an explanation for the greater efficacy of morphine [46]. Based on our work, we surmise the difference can be explained by considering the various types of opiate receptor subtypes. Morphine exhibits a K_d of approximately 20 nM for the μ_3 receptor whereas, this receptor is largely insensitive to enkephalins until a concentration of 1 mM is reached when binding may occur [22,32], supporting a role for morphine and not opioid peptides in this phenomenon. This hypothesis is also noted by the fact that in these cells β-endorphin is without effect while this opioid peptide effect still occurs in knock out mice deficient in this peptide [46,51,52]. Interestingly, a κ-opioid ligand also exert preconditioning protection (dynorphin A [1–8]; [46]), suggesting multiple opioid peptide involvement.

In addition, it appears that the opioid preconditioning phenomenon can be coupled with other cellular signaling processes, supporting that this action may be an intended function of the endogenous cardiac signaling system. The opioid activation is mediated via protein kinase C [38]. It also occurs through opening

Table 1. Displacement of ^{3}H-dihydromorphine (DHM; nmol-L^{-1}) by opioid ligands in various human tissue membrane suspensions.

LIGAND	Vein	Artery	Atria-E
δ-agonist			
DPDPE	>1000	>1000	>1000
μ -agonist			
Endomorphin 1	>1000	>1000	>1000
Endomorphin 2	>1000	>1000	>1000
Orphanin FQ (N)	>1000	>1000	>1000
DAMGO	>1000	>1000	>1000
Fentanyl	>1000	>1000	>1000
Dihydromorphine	19 ± 2.1	21 ± 3.0	24.3 ± 3.1
Morphine	23.4 ± 2.4	21.3 ± 2.5	20.1 ± 2.0
M6G	73.5 ± 4.1	69.0 ± 3.9	74.4 ± 4.7
M3G	>1000	>1000	>1000
κ-agonist			
Dynorphin 1-17	>1000	>1000	>1000
50-488h	>1000	>1000	>1000
U69-593	>1000	>1000	>1000
Antagonists			
Naltrexone	34 ± 5.1	37 ± 3.8	32.6 ± 3.5

* One hundred per cent binding is defined as bound ^{3}H-DHM in the presence of 10 μM dextrorphan minus bound ^{3}H-DHM in the presence of 10 μM levorphanol. IC_{50} is defined as the concentration of drug which elicits half-maximal inhibition of specific binding. The mean SD. for three experiments is given. DPDPE = (D-Pen^2, D-Pen^5)-enkephalin; DAMGO = [Tyr-D-Ala^2, Gly-N-Me-Phe^4, Gly(ol)^5)-enkephalin] M6G = morphine 6 glucuronide; M3G = morphine 3 glucuronide.

the ATP-sensitive potassium channel, long associated with this type of phenomenon [53].

Recently, in rats, we demonstrated that morphine, not opioid peptides, can release NO from median eminence fragments in a naloxone sensitive manner [54]. This phenomenon was further coupled to CRF and gonadotropin releasing hormone (GnRF) release, demonstrating a neurovascular modulatory process since it was the vascular endothelium that contains the μ opiate receptor transcript, which is coupled with NO release.

Morphine in the Heart

In 1976 it was proposed that opiate alkaloids, i.e., morphine, may be an endogenous signal molecule [2]. Since that time, morphine has been found in mammalian, amphibian and invertebrate tissues, including neural and immune tissues see [22]. In regard to rat heart, morphine immunoreactivity following HPLC was found [19], suggesting, within the limits of this identification, that it is present.

In a recent report we conclusively demonstrate the presence of endogenous morphine in human atria via HPLC coupled with matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass determination [55]. In this study, morphine was identified in human heart tissues by reverse phase HPLC using a gradient of acetonitrile following liquid and solid extraction, and comparison to an authentic standard. Experiments were carefully performed to prevent exogenous morphine contamination. The morphine extracted from heart tissue was identical with that of a morphine external standard. This finding was repeated in heart tissue from five patients, all of which were never exposed to exogenous morphine. The electrochemical detection sensitivity of morphine in biological samples is at the 80 picogram level. The concentration of morphine was determined using the Chromatogram Manager 3.2 (Millemmium, Waters, Milford, MA) and extrapolated from the peak-area calculated for the external standard. The average concentration of morphine in the 5 samples was 106.28 ± 61.58 ng/gm wet weight atria tissue. Chromatogram of vein extracts did not show a peak at the retention time of morphine. Blank runs between morphine HPLC determinations did not show a morphine residue. All the fractions corresponding to morphine blank runs which were sent for mass spectrometric analysis returned negative.

HPLC coupled with the MALDI-TOF further characterized the morphine in human atria tissues. The molecular mass attributed to protonated morphine is 285.7 Da, instead of the calculated molecular mass of 286.1 Da. The 0.4 Da difference in mass between the mass spectra data and the theoretical data is due to the normal variation in the detection of this machine. There is also an isotope, hydrogen, appearing in the mass spectra (m/z 286.6,). The molecular mass found for the MS standard (2 molecules of morphine linked by a sulfate group; theoretic molecular weight: 668. 8Da) was 285.7 Da, corresponding to a single molecule of morphine (M, 286.1 Da), derived from the break down of the linkage between the morphine and the sulfate group. The lowest amount of this material detected is 0.5 ng with a ratio signal/noise (s/n) of 5.2**.** MALDI-TOF analysis confirmed the presence of morphine in heart tissue, but not in saphenous vein pieces – in agreement with the HPLC analysis. The HPLC fraction of a vein-negative run did not show presence of morphine in the MALDI-TOF detection.

Besides heart tissue, morphine has been demonstrated in mammalian brain and adrenal gland as well as in human plasma [1,5,7,9,14,20,22,23,27,56]. As noted earlier, studies from our laboratory have demonstrated the presence of an opioid-peptide insensitive and opiate alkaloid selective receptor, designated μ_3 [28], on human endothelia as well as other cell types

Figure 1. Comparison of the putative mu conserved sequence from human internal thoracic artery and atria with the known human brain mu opioid receptor sequence. Both negative and positive strands were sequenced, however, only the sequence from the negative strand is shown. Sequence obtained from all of the tissues, were identical. The tissues were washed extensively with PBS to limit the amount of leukocyte contamination.

see [22]. In vascular endothelia obtained from the saphenous vein, internal thoracic artery, and human atria, this receptor is also present [25,30–32,57,58]. These reports also document that this receptor in human endothelia is coupled with constitutive nitric oxide (NO) release.

Recently, we have demonstrated that this receptor, using primers derived from the human neuronal μ1 opiate receptor via RT-PCR, is expressed in human endothelia [33] (Figure 1). Sequence analysis of the RT-PCR product revealed 100% identity with the neuronal human μ1 receptor. Only one pair of μ-specific primers amplified a transcript of the expected size for the μ receptor (441 bp). Furthermore, tissues denuded of their endothelial layer, were found not to express these transcripts. The other primer pairs that spanned the coding region of the μ1 receptor did not yield specific PCR products, indicating μ_3 may be a splice variant [33]. We further showed that pretreatment of human internal thoracic artery and cardiac atrial endothelium with the proinflammatory cytokines interleukin-1-α and -β led to a significant increase in both the expression of the μ transcript and in morphine-stimulated NO release measured amperometrically [33]. Thus, human atria contain a receptor that can selectively recognize endogenous morphine.

Conclusion

At present, it is premature to speculate as to whether atria have the ability to synthesize morphine. In this regard, we surmise the material may come from the brain/hypothalamic area and/or the adrenal gland see [20,22] since plasma contains morphine [22]. This suggests that the atria have the ability to selectively absorb this material whereas, saphenous vein does not. Additionally, we further surmise that atria morphine via NO may serve to down regulate the excitability of this tissue see [30] given the heart's high performance state as compared to that of the saphenous vein, a passive resistance conduit. Furthermore, as discussed earlier in ischemic preconditioning, morphine may selectively function as a regulatory molecule via NO in this phenomenon. The binding displacement data on vascular endothelial cells is suggestive of additional novel receptor sites specific for opiate alkaloids as noted by the significant difference in the morphine 6 glucuronide displacement of dihydromorphine (table 1). This in turn suggests that various opiate alkaloids may also serve in a hormonal capacity, making this a "family" of neuroimmune and neurovascular signaling molecules. Taken together, recent studies are demonstrating that

opiate alkaloids may represent a new hormone family and the vascular tissues, in part, may be its intended peripheral target.

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