MINIREVIEW

Cardiac Opioids (44507A)

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Abstract. **Opioid peptides have long been considered as neuropeptides or neurotransmitters. The more recent discovery of these same peptides in non-neuronal tissue suggests that the peptides may have autocrine, paracrine, or endocrine functions as well. The opioid peptides, enkephalins, dynorphins, and endorphins, have been found in isolated cardiac myocytes and heart tissue. This review will cover the recent literature on opioid peptides in respect to cardiac distribution, biochemistry, and function.**

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Opioid peptides constitute a large group of small proteins that interact with cell membrane receptors similarly to opiate alkaloids, morphine and heroin. Opiate alkaloid derivatives are extensively used for analgesia and anesthesia. The original opioid peptide families are enkephalins, dynorphins, and endorphins. Representative peptides from these three opioid peptide families have been found in the heart. Three different opiate receptors have been cloned and sequenced: mu (μ), delta (δ), and kappa (κ). Evidence for cardiac opiate receptor activity has been provided as well. This review will focus on the evidence for cardiac myocyte production of enkephalins and the possible functions that they may have in the heart.

Enkephalins were first characterized in the brain and adrenal gland (1, 2). Enkephalins and their receptors are found both centrally (brain and spinal cord) and peripherally (autonomic nerves and ganglia, gut, and endocrine tissues). Many central sites with peptide content and opioid receptors are cardiovascular control centers. These include the preoptic nucleus; hippocampus; periacqueductal gray, dorsal motor nucleus of the vagus; hypothalamus; and nucleus tractus solitarius (3). Enkephalins have also been localized to many autonomic ganglia and nerves, including the stellate ganglia and vagus (4–7). However, opioid peptides are also contained in non-neuronal tissue. Enkephalins have been found in the spleen, heart, vas deferens (8), stomach, lung, pancreas, and liver (5). Therefore, enkephalins are in optimum position to modulate cardiovascular function.

The amino acid sequence of methionine-enkephalin is tyrosine-glycine-glycine-phenylalanine-methionine (met-enk). The proenkephalin sequence contains four copies of the pentapeptide met-enk, one of leu-enkephalin, and two extended forms of met-enk (met-enk-arg^6-phe^7 and met-enk-arg^6-gly^7-leu^8) as shown in Figure 1. Pairs of basic amino acids mark these small peptides for cleavage from the precursor. Proenkephalin is processed by endoproteolytic enyzmes termed prohormone convertases, which recognize and cleave at dibasic amino acid sites. Initial proenkephalin processing starts before transport to the Golgi network and is rapid. Later processing requires an acidic environment distal to the Golgi network (9). Proenkephalin has a fast cleavage to peptide B, and slower cleavages yield other intermediate sized products that are cleaved ultimately to the penta- to octapeptides (10). The different molecular-weight end products found in diverse tissues (muscle, neural, endocrine) may be due to variations in the cleavage sequence and local enzymatic conditions for processing.

Neuronal tissues (brain, ganglia, and adrenal medulla) contain met-enk to met-enk-arg-phe in a ratio of 4:1 as is expected from the sequence of the precursor and complete processing. However, proenkephalin processing in peripheral tissue, such as the heart and lung, is not complete.

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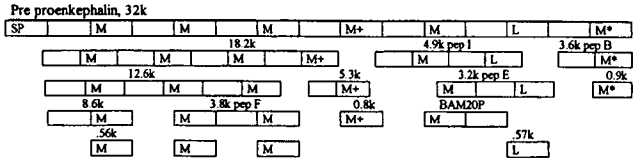

Figure 1. Preproenkephalin peptide sequence and processing. (Adapted from Ref. 2.) The molecular weights are for the peptide beneath the number. SP, signal peptide; M, met-enk; M +, met-enk-arg-gly leu; M*, met-enk-arg-phe; L, leu-enkephalin.

Met-enk-arg-phe concentration is greater than met-enk in both the heart (5, 11) and lung (12). Similarly, prodynorphin was sequenced and shown to have three leucine-enkephalin copies but multiple molecular-weight products (2). As with the enkephalins, heart tissue and isolated cardiac myocytes contain larger molecular-weight, immunoreactive dynorphin (13, 14). Therefore, the heart appears to process and use opioid peptides differently from neuronal tissue.

Opioid Peptides in the Heart

Cardiac tissue and isolated cardiac myocytes contain dynorphins and prodynorphin mRNA (13, 14), enkephalins and proenkephalin mRNA (15, 16), and β-endorphin and pro-opiomelanocortin mRNA (17–19). In addition, physiological conditions regulate the message and the peptide products. Myocardial infarction, aging, hypertension, heart transplantation, and cardiomyopathy all lead to increased enkephalins or proenkephalin mRNA in the heart (20–24). However, the peptide and message content are not tightly correlated. The message may be high whereas the peptide content is low. The ability of the peptides to regulate transcription or translation has not been addressed. The opioid mRNA studies were all conducted in rodents and lacked chromatographic analyses of the peptides produced by the heart. Therefore, larger molecular weight opioids may have been present in the heart tissue extracted but not quantitated. The function of the larger opioid peptides is unknown. Perhaps they serve as a ready reservoir or precursor for cleavage to the smaller opioid receptor–active peptides when required or are released to supply small peptides when arriving for cleavage at the site of action. Further studies are needed to identify the peptides produced and the signal for increased enkephalin production and release in the myocardium.

Extraction of mammalian hearts for radioreceptor-, radioimmunoassay, and immunohistochemistry proved that the myocardium contains enkephalins (11, 25). An antibody raised against the proenkephalin carboxyl terminus, met-enk-arg-phe (Fig. 1), was used to demonstrate greater enkephalin content in the ventricles than the atria. However, met-enk is uniformly distributed in all four chambers. Unexpectedly, met-enk-arg-phe content is greater than met-enk in all heart chambers. Indeed, the met-enk-arg-phe distribution is opposite to that of catecholamine content, an index of sympathetic innervation. In addition, immunohistochemical photomicrographs revealed a diffuse met-enk-arg-phe staining in the cardiomyocytes with more intense staining at the intercalated disc (11). The high met-enk-arg-phe:met-enk ratio and the difference in atrial:ventricular distribution of the two peptides led to the conclusions that i) proenkephalin is contained predominately in the myocardium rather than in neuronal tissue; and ii) proenkephalin is processed differently in the heart versus neuronal tissue. These studies also indicated species diversity in distribution of proenkephalin products in the heart. For example, in the pig and cat heart, met-enk content is greater in the atria than ventricles (11). However, met-enk-arg-phe distribution is similar in the dog, pig, and cat hearts: in all three species, the ventricles contain more met-enk-arg-phe than the atria. This may indicate that in the pig and cat there is a larger enkephalin content in the atrial nerves than in the dog or that there is even less processing to the final pentapeptide product in the ventricles of the pig and cat.

Additional experiments revealed canine cardiac enkephalin and catecholamine content changed with physiological and pharmacological manipulations. Acute and chronic manipulations changed cardiac enkephalin in similar ways. Acute ganglionic blockade (8) and chronic renovascular hypertension (26) increased canine cardiac opioid and catecholamine content compared with control. Both chronic exercise and acute hemorrhagic hypotension decreased cardiac met-enk and catecholamine content and increased ventricular met-enk-arg-phe (25, 27). However, these last two stimuli had opposite effects on atrial met-enk-arg-phe-immunoreactivity. Hemorrhage increased atrial met-enk-arg-phe whereas exercise training decreased left atrial met-enk-arg-phe. Further chromatography revealed that the hemorrhage-induced increase in atrial met-enk-arg-phe was due to increased peptide B whereas the ventricular increase was due to more proenkephalin content. The possible similarity between acute ganglionic blockade and chronic hypertension is the depressed autonomic function. Acute ganglionic blockade is a direct depression of autonomic function, whereas hypertension causes increased sympathetic tone resulting in downregulation of adrenergic receptors. Hypotension and exercise training both increase sympathetic stimulation to the heart. Acute ganglionic blockade and hypotension are associated with lowered blood pressure but both produced opposite effects on cardiac enkephalin content. Therefore, the change in autonomic stimulation and not the change in blood pressure may be more important in changing enkephalin production and processing. Chronic exercise training decreased met-enk content in dog but not in pig hearts (28), again showing that species differences are seen in endogenous opioid peptide systems. Changes in opioid peptide tissue content may be due to changes in synthesis, processing, release, uptake, or degradation. Further studies are needed to determine which of these mechanisms are responsible for changes in cardiac opioid content.

The enkephalin-immunoreactive products detected by the met-enk-arg-phe antibody include proenkephalin (31

kDa), peptide B (3.6 kDa), and met-enk-arg-phe (0.9 kDa) (Fig. 1). Previous data are from unchromatographed samples. Subsequent experiments were analyzed after size exclusion chromatography separated these products. Mateo *et al.* (27) showed that all these possible proenkephalin products are found in the dog heart. The peptide B fraction contains the most immunoreactivity. Thus, the ventricular:atrial difference is maintained mostly by the larger molecular-weight fractions, indicating incomplete processing of the precursor.

Hypotension significantly increased canine cardiac contents of proenkephalin and peptide B without affecting the met-enk-arg-phe content in all heart chambers. This increase in tissue precursors with no change in product suggests several possible explanations: (i) synthesis increased with more release of product, met-enk-arg-phe; (ii) synthesis increased with more release of product than precursors; or (iii) synthesis increased with more release of precursors and product with a rate-limiting step in the final processing to met-enk-arg-phe (27). However, the increase in plasma peptide B was less than in the increase in plasma met-enk-arg-phe in combination with the increase in cardiac tissue. This suggests that a large capacity for proenkephalin synthesis and processing is present in the heart and that the processing step from peptide B to met-enk-arg-phe may be rate limiting. In fact, Rostovtsev and Wilson (9) showed that the initial processing to peptide B was faster than a second phase of processing to the smaller peptides. The possibility of changes in precursor processing versus changes in peptide release need to be investigated.

Opioid Peptides and Cardiac Function

Opiate receptor stimulation causes functional changes in the heart and cardiomyocytes. In fact, all of the subtype selective opiate receptor agonists have effects on the heart. For instance, studies using agonists have revealed the following. Only μ agonists inhibited neurally stimulated contractile response in the guinea-pig atrium, but κ and δ receptor agonists were effective in this regard in the ventricle (29, 30). Others found in guinea pig and dog, respectively, κ-receptor–mediated inhibition of stimulated norepinephrine release measured across the heart *in vitro* (31) and *in vivo* (32). Gu *et al.* (33) further demonstrated that the inhibition of stimulated norepinephrine release was not due to stimulated uptake in the dog. In fact, dynorphin acting at a receptor that was not typical in the binding of other κ-agonists inhibited the uptake of norepinephrine in rat heart (34). Therefore, assuming similar control mechanisms in the dog and rat, the demonstration of opioid inhibition of catecholamine release in the face of opioid inhibition of catecholamine uptake, which should increase catecholamine release, is highly significant.

In the isolated perfused rat heart, a κ-selective agonist was the most potent at causing arrhythmias (35), and dynorphin$_{1-13}$-induced arrhythmias, in this model, were reduced by chronic morphine treatment *in vivo* (36). This indicates that chronic opiate treatment downregulated opioid receptors involved in the dynorphin-induced arrhythmias. The κ agonist (U50,488) also caused a reduction in heart rate and contractility, which were prevented by chronic κ agonist treatment (37). The inference can be made that chronic morphine treatment altered κ receptor function. Morphine is thought to act primarily through μ receptors; therefore an alteration of κ receptor function is possibly indirect. These findings also demonstrate that acute and chronic effects of opioids are different. Adaptations occur with chronic elevation of opiates; therefore, pathological changes that elevate endogenous opioid peptides should cause some adaptive changes. Chronic disease states need to be characterized as to opioid peptide changes to understand these phenomena better.

Opioid peptide activation of their receptors result in primarily inhibitory effects on autonomic nerve traffic, presynaptic release of neurotransmitters, and postsynaptic neurotransmitter function (38–40). Figure 2 shows the opioid receptors coupled to various second messengers: ion channels, adenylate cyclase, and inositol phosphate turnover (41–47). Thus opioids can modulate contraction, action potentials, or exocytosis. Opioid peptide effects in this regard are often studied in neuronal tissue or cultured cells. Interestingly, in a cation channel that is similar to the ion channel of the cardiac SA node, opioids reversed the forskolin stimulation of current (46). In the superior cervical ganglion, met-enk-arg-phe reduced the stimulation-induced increase in Ca^{++} accumulation, thus, decreasing acetylcholine release (45). The cloned μ-opioid receptor couples to both adenylate cyclase and phosphatidyl inositol turnover to reduce stimulated cAMP and IP_3 levels (44). Both μ- and δ-opioid receptors were shown to inhibit N- and P/Q-type Ca^{++} channels in neurons from the nucleus tractus solitarius (45). Conversely, Williams *et al.* (41) demonstrated that enkephalin could increase the potassium conductance in

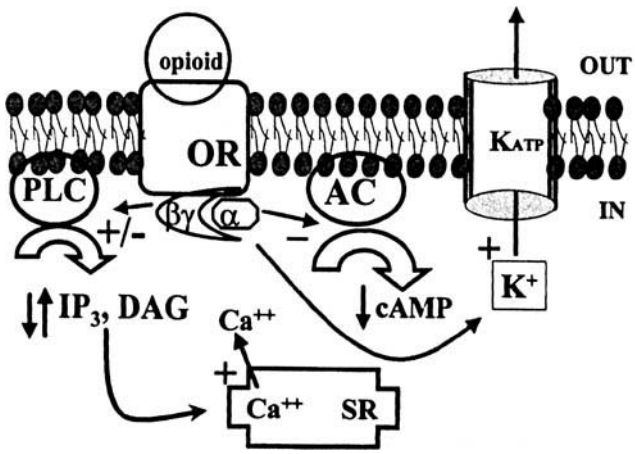

Figure 2. Diagrammatic representation of the opioid receptor and its second messengers. The opioid receptor (OR) is a molecule with seven transmembrane-spanning regions linked to G proteins. PLC, phospholipase C; IP_3, inositol (1, 4, 5) trisphosphate; DAG, diacylglycerol; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; SR, sarcoplasmic reticulum; K_{ATP}, potassium-ATP channel.

neurons from the locus coeruleus. More relevant to this review, δ and κ-opioid selective agonists increased intracellular Ca^{++} in ventricular myocytes primarily through depletion of sarcoplasmic reticulum Ca^{++} (48, 49). In addition, the twitch amplitude of rat ventricular myocytes was diminished by the presence of opioid agonists. Conversely, δ-selective agonists increased the contractility, cAMP content, and Ca^{++} uptake into cultured chick cardiac myocytes (50). Taken together these data show opioid regulation of cardiac function both directly and through autonomic control mechanisms. Thus endogenous opioid peptides play an important role in the regulation of cardiac function.

Further support for opioid regulation of cardiac function through the autonomic nervous system has been reported. Met-enk-arg-phe is a promiscuous opioid peptide that binds with high affinity to both δ and κ receptors and with lesser affinity to μ receptors (51). In addition, preliminary data show that cardiac homogenates degrade met-enk faster than met-enk-arg-phe (52). This may explain why met-enk-arg-phe is three times more potent than met-enk in inhibiting vagally induced bradycardia and negative inotropic responses in anesthetized dogs (53, 54). Also, met-enk-arg-phe at picomolar infusion rates inhibits vagal bradycardia without affecting the response to methacholine. This implies that met-enk-arg-phe inhibited the release of acetylcholine either at the parasympathetic ganglion or at the SA node (53). Vagal stimulation–induced bradycardia in addicted dogs was also profoundly reduced, suggesting altered parasympathetic control of heart rate in this model (55). The vagal bradycardia in control animals was unaltered by the administration of acute morphine or by the administration of the opiate antagonist, naloxone. Similarly, dynorphin inhibited the cardiac response to sympathetic nerve stimulation by decreasing the release of norepinephrine (36). These data indicate that endogenous opioids can change the heart's responsiveness to vagal and sympathetic stimulation. Furthermore, changes in opioid or cholinergic receptor systems may have been induced by chronic morphine treatment. The bilateral carotid occlusion-induced increase in arterial pressure was also substantially reduced in chronically morphine-treated dogs while under anesthesia. Naloxone administration resulted in an increase in arterial pressure in chronically treated dogs that was not accompanied by a reflex decline in heart rate (55). These results suggest a blunted baroreflex function because of morphine addiction. Again, the impaired response seems to result from chronic morphine treatment and not from its acute influence. These data show that opioids inhibit both sympathetic and parasympathetic stimulation of the heart. Could an abnormality in opioid peptide processing or release cause the same type of change in vagal and baroreflex function? Do pathological conditions such as hypertension result in opioid peptide abnormalities?

Since opioids inhibit the autonomic nervous system, does autonomic stimulation change opioid peptide content or release? Is there a role for opioid peptides in "cross talk" between the sympathetic and parasympathetic nervous system? Opioids are known to inhibit the release of catecholamines both *in vitro* and *in vivo* (35, 36, 56) resulting in decreased effector responses to nerve stimulation. In fact, adrenergic second messenger stimulation increased enkephalin production in isolated cardiomyocytes (21). This strongly suggests a role for enkephalins in autonomic "cross talk."

Opiate Receptors and the Heart

Most studies infer the activity of endogenous opioids at cardiovascular opiate receptors by the action of opiate receptor antagonists. Development of selective opiate receptor antagonists has furthered this research. Selective δ-receptor antagonism but not μ-receptor antagonism increased blood pressure, cardiac output, contractility, and blood flow to the heart, kidneys, GI tract, and skeletal muscle in conscious dogs with right heart failure (57). Naloxone, a nonselective opiate antagonist, selectively increased local cardiac ventricular contractile force and O_2-consumption when injected intracoronary, but not when infused systemically at a similar dose. Thus, cardiac opioid effects were displayed in the anesthetized dog (58). In the dog and rat, but not the pig, naloxone reduced the incidence and severity of cardiac arrhythmias and mortality during coronary occlusion and reperfusion (59, 60), showing the species differences in myocardial function. The blockade of opiate receptors increases the dog heart's response to β-adrenergic stimulation (61) suggesting an endogenous opioid effect to decrease cardiac adrenergic stimulation. Conversely, adrenergic stimulators increase rat cardiac opiate receptor number and affinity (62). Thus, assuming that in the rat as well as in the dog, opioids inhibit β-adrenergic catecholamine release, and adrenergic second messengers increase opioid receptor number and affinity, a possible feedback loop exists between these two systems. Naloxone infusion significantly blunts canine baroreflex function mediated by both the parasympathetic and sympathetic nervous systems (63). Cardiovascular effects of proenkephalin peptides in the rat are mediated by peripheral μ-opioid receptors and change central nervous system activity *via* vagal afferents (64). These results implicate the action of endogenous opioid peptides but do not prove it, since any receptor antagonist may have nonreceptor effects at other sites (65, 66). However, endogenous opioid peptides (enkephalins or dynorphins) acting *via* cardiac opiate receptors may play an important role in the reciprocal interactions between sympathetic and parasympathetic cardiac control. More direct evidence of peptide action (peptide release, peptide mimicry of effect) is needed in this regard.

Controversy, complicated by species differences, exists about the type of opiate receptor present in the heart. Classical opiate receptor binding studies on cardiac tissue have been carried out only in rodents. In this regard, rodent cardiac κ- and δ-opiate receptors are distributed more to the right side than the left and are more concentrated in the atria

than the ventricles (53, 62, 67). Physiologic manipulations can regulate the cardiac opiate receptors. Hemorrhagic hypotension caused a reversible decrease in opiate binding (67). Spontaneously hypertensive rats had high affinity cardiac δ-receptors whereas their normotensive controls had both high- and low-affinity receptors. The mechanism of these changes is unknown. In both experiments (hypotension and hypertension) a greater adrenergic input to the heart is hypothesized. Opioid peptides and their receptors are present in the heart, but chronic pathological effects on the opioid peptide system, in a large animal model routinely used for cardiovascular functional studies, have not been determined.

Opioid Peptides and Preconditioning

Recent data have revealed that opioid peptides are involved in the phenomenon termed cardiac preconditioning. Preconditioning is the ability of a short insult (ischemia or hypoxia) to protect the heart from damage due to a subsequent prolonged insult. The result is a smaller infarct size in proportion to the area at risk. The first indication of this was the finding that naloxone (nonselective opiate receptor blocker) blocked the preconditioning effect (68). Subsequently, Schultz *et al.* (69, 70) reported the opioid receptor involved in the preconditioning response was in the periphery and was most likely due to activation of the δ opioid receptor. Schultz *et al.* (71) further demonstrated that the δ_1 but not the μ or κ opioid receptor subtype was responsible for ischemic preconditioning. These studies all relied on the activity of selective antagonists. Induction of preconditioning with morphine and a δ_1-opioid receptor agonist further proved that endogenous opioid peptides are involved in preconditioning in rats (70, 72). In addition, the δ_1 agonist effect to produce preconditioning was abolished by glibenclamide (K_{ATP} channel blocker) and pertussis toxin, demonstrating the involvement of K_{ATP} channels and G proteins. These data show that opioid peptides in the heart have beneficial cardiac-specific actions.

Opioid Peptides and Hypertension

Opioid peptides are known to modulate sensitivity of the vasculature to adrenergic agents. Hypertension changes the importance of these effects and autonomic nervous system function. For instance, μ-agonists inhibited electrically stimulated vasoconstriction without changing basal tone only in isolated arteries from hypertensive rats. Conversely, κ-agonists directly increased vascular tone in both normotensive and hypertensive isolated arteries. However, aging eliminated the κ-agonist-induced increase in vascular basal tone only in the hypertensive animals (73). In isolated ventricular myocytes, a κ agonist attenuated the increase in Ca^{++}_i induced by electrical stimulation, norepinephrine, and forskolin (74) without affecting basal Ca^{++}_i. The stimulated increase in Ca^{++}_i from spontaneously hypertensive rat (SHR) ventricular myocytes was attenuated compared with Wistar Kyoto (WKY) controls. In addition, in older SHR myocytes the ability of the κ agonist to attenuate the stimulated response was decreased. The alterations in activation of adrenergic and opioid receptors was not secondary to the elevated blood pressure since normalization of blood pressure by pharmacological treatment did not reverse the difference between SHR and control (74). These results demonstrated the ability of the opioids to modulate both vascular and cardiac responsivity to autonomic stimulation.

Aging and hypertension were accompanied by change in opioid peptide and opioid mRNA contents (75–77). Cardiac met-enk content decreased with age in both normotensive and hypertensive rats. However, the increase in cardiac proenkephalin mRNA with age was greater in hypertensive rats (75). In addition, the hypertensive rats displayed a circadian variation resulting in increased cardiac met-enk at the beginning of light and dark cycles as compared with normotensive controls. These authors hypothesized a cyclic or transitory upregulation of DNA transcription and/or derepression of mRNA translation occurring to a greater extent in hypertensive animals than in normotensives. However, another interpretation is a possible change in processing of the precursor, proenkephalin, into products as a regulatory site at the different times. Measurements of not only plasma precursor/product cardiac release but also tissue precursor/product content should help in resolving processing versus transcription/translation changes.

Sympathetic nerves release not only catecholamines, but also peptide co-transmitters such as enkephalins, dynorphins, and neuropeptide Y. These co-transmitters can have profound effects on the activity of the catecholamines at the neuroeffector junction and on presynaptic mechanisms to modulate release. Presynaptic α_2-inhibition of norepinephrine release is decreased in young spontaneously hypertensive rats (SHR) causing increased norepinephrine release. Presynaptic β_2-stimulation of norepinephrine release is greater in SHR than control. At high epinephrine concentrations the α_2-inhibition is seen and at low epinephrine concentrations the β_2-stimulation is seen. Epinephrine is taken up by adrenergic nerve terminals and released as a co-transmitter with norepinephrine. Met and leu-enkephalin are also co-transmitters in postganglionic nerves. The enkephalin inhibitory effect on norepinephrine release is attenuated in SHR suggesting insufficient regulation by opioid peptides (78).

The finding of hypoalgesia in hypertension resulted in the first linking of opioid peptides and this disease state. In fact, recent correlation of hypoalgesia to hypertensive parental history in normotensive humans (79) suggests that altered opioid peptide function predates hypertension development and may therefore be implicated in the etiology of essential hypertension. Opiates are drugs used for analgesia and anesthesia in cardiovascular emergencies and surgery. The endogenous opioid peptides have profound effects on the action of autonomic neurotransmitters at both pre- and postsynaptic sites. Therefore, a better understanding of the endogenous opioid peptide interactions in the cardiovascu-

lar system will improve therapeutic strategies for clinical use of opiates. Cardiac opioid peptides probably participate in multiple functions (modulation of autonomic signaling, ion flux, immune function, hypertrophy, etc.). Future experiments will need to be done to elucidate the role of these peptides in cardiovascular function. Are they released into the plasma for an endocrine function or are they leaked into the plasma after serving a paracrine or autocrine function?

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