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CARDIOVASCULAR EFFECTS OF ENDOGENOUS OPIATE SYSTEMS

John W. Holaday

Neuropharmacology Branch, Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington D.C. 20012

INTRODUCTION

Opiates are among the oldest pharmacological substances known to man; their analgesic, euphoric, and addictive effects have been traditional focal points for opiate research. Without doubt, the cardiovascular effects of opiates have also been apparent to the user or abuser of opiate substances for several centuries. Persons seeking analgesic, euphoric, or antidiarrheal actions from opiate alkaloids have probably noted dizziness upon sudden standing due to the orthostatic hypotension these substances produce. Historically, however, scientific study on the cardiovascular responses to administered opiates has lagged behind other areas of opiate research. This is understandable as, relative to doses required for their analgesic actions in humans, the cardiovascular effects of opiates are less noticeable and, indeed, undesirable.

Early studies established that cardiovascular responses to morphine varied among species; both autonomic and histamine-releasing properties contributed to their hypotensive and bradycardic actions (1–5). Generally speaking, morphine was shown to produce prominent effects upon brainstem and hypothalamic centers that resulted in increased parasympathetic and decreased sympathetic tone (3–6). These effects upon autonomic outflow caused a depression of both heart rate and blood pressure.

A resurgence of interest in opiate-cardiovascular interactions followed the discovery of endogenous opiate systems (for review see 7). A family of opioid peptides were found to be located at sites suggesting an autonomic action (8); also, opiate receptors were shown to be densely distributed in the brainstem and hypothalamus in close proximity to cardiovascular centers as well as endogenous opiate pathways (9, 10). These anatomical findings

were predictive of functional interactions. Moreover, administration of opioid peptides intracerebrally, intravenously, or applied directly upon brainstem areas produced potent cardiovascular responses (11–15). Lastly, the demonstration that pathophysiological states such as circulatory shock not only were reversed by opiate antagonists (16–18) but were also accompanied by elevations of circulating opioid peptides (19–21) has added further credibility to the hypothesis that endogenous opiate systems play a functional role in cardiovascular regulation.

As is often the case, simple initial observations have yielded to a more complex picture regarding the sources and sites of opiate-cardiovascular interactions. Endogenous opiate peptides are a heterogeneous mixture of several molecules (Table 1). Although they share some important structural similarities, their differential synthesis, distribution, and release in central and peripheral tissues suggests that endogenous opiates may function as neurotransmitters, hormones, or neuromodulators with effects upon a variety of physiological and behavioral systems involved in regulating blood flow. Their actions are mediated not by a single opiate receptor, but by a family of receptors that may recognize different opiates in different ways to affect variable, sometimes opposing cardiovascular actions. The presence or absence of concomitant anesthetics can change a bradycardic, hypotensive opiate effect into tachycardia and/or hypertension. Other factors such as pH, temperature, and respiratory variables
responses to opiate agonists or antagonists.

Although such complexities may seem to be insurmountable obstacles in developing a clear picture of opiate-cardiovascular interactions, they are not unique to these substances and some generalizations can be made. This review is not intended as an exhaustive compilation of the autonomic, pharmacological, or physiological evidence of opiate-cardiovascular relationships. Several review articles contain specific information in this regard (7, 22–24). Rather, I attempt to provide a theoretical framework within which to construct novel insights into this rapidly developing area, and discuss certain pitfalls in opiate-cardiovascular studies.

BRIEF REVIEW OF CENTRAL AUTONOMIC-CARDIOVASCULAR PATHWAYS

Information regarding peripheral hemodynamic changes is detected by mechanoreceptors in the major arteries and relayed via vagal afferents to the brainstem. Stimulation of chemoreceptors, such as pulmonary "J" receptors found in the alveoli of the lung adjacent to pulmonary capillaries (25), can also result in the detection of changes in cardiorespiratory variables, which are likewise relayed via the ninth and tenth cranial nerves to

Table 1 Major endorphins and their amino acid structures

Name	Amino acid sequence	Molecular weight
Naturally occurring opioid peptides		
Met-Enkephalin	Tyr Gly Gly Phe Met	573.8
Leu-Enkephalin	Tyr Gly Gly Phe Leu	555.7
α-Neo-Endorphin	Tyr Gly Gly Phe Leu Arg Lys Arg Tyr Pro Lys	1228.6
Met-Enkephalin-Arg^6-Phe^7	Tyr Gly Gly Phe Met Arg Phe	877.1
Dynorphin	Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Asn Gln	2147.8
α-Endorphin	Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr	1746.2
β-Endorphin (human)	Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr Leu Phe Lys Asn Ile Ile Lys Asn Ala Tyr Lys Lys Gly Glu	3465.6
Kyotorphin	Tyr Arg	337.4
β-Casomorphin	Tyr Pro Phe Pro Gly Pro Ile	790.0
Dermorphin	Tyr d-Ala Phe Gly Tyr Pro Ser	804.0
Selected analogs of opioid peptides and their reported receptor selectivity		
d-Ala^2-Met-enkephalin-amide (DAME: nonselective agonist)	Tyr d-Ala Gly Phe Met NH_2	586.8
d-Ala^2-d-Leu^5-enkephalin (DADLE: δ-agonist)	Tyr d-Ala Gly Phe d-Leu	569.7
d-Ala^2-MePhe^4-Met(o)ol-enkephalin (FK 33824: μ-agonist)	Tyr d-Ala Gly N-Me-Phe Met(o)ol	605.8
β-casomorphin (1–4) amide (morphiceptin: μ-agonist)	Tyr Pro Phe Pro NH_2	521.6
diallyl-Tyr^1-(CH_2S)-Phe^4-Leu-enkephalin (M 154, 129: δ-antagonist)	N,N Diallyl Tyr Gly Gly (CH_2S)-Phe Leu	998.0
des-Tyr^1-Leu-enkephalin	Gly Gly Phe Leu NH_2	401.5
FMRF amide Molluscan cardioexcitatory neuropeptide	Phe Met Arg Phe NH_2	598.8

central autonomic centers. In the brainstem, several nuclei are integrally related in the functional maintenance of cardiovascular homeostasis (26, 27). The diencephalon also functions to integrate autonomic responses; autonomic nuclei in this region receive information from the brainstem as well as from limbic, cortical, cerebellar, reticular, and other brain regions [Figure 1, (26, 27)]. The central integration of these inputs ultimately results in an orchestration of autonomic cardiovascular responses through alterations in variables such as heart rate, cardiac contractility, peripheral resistance, and adrenal medullary outflow.

With reference to the parasympathetic pathways, the nucleus tractus solitarius (NTS) is the primary CNS synapse of the baroreflex and chemoreflex arcs (Figure 1). This nucleus directly innervates the nucleus ambiguus

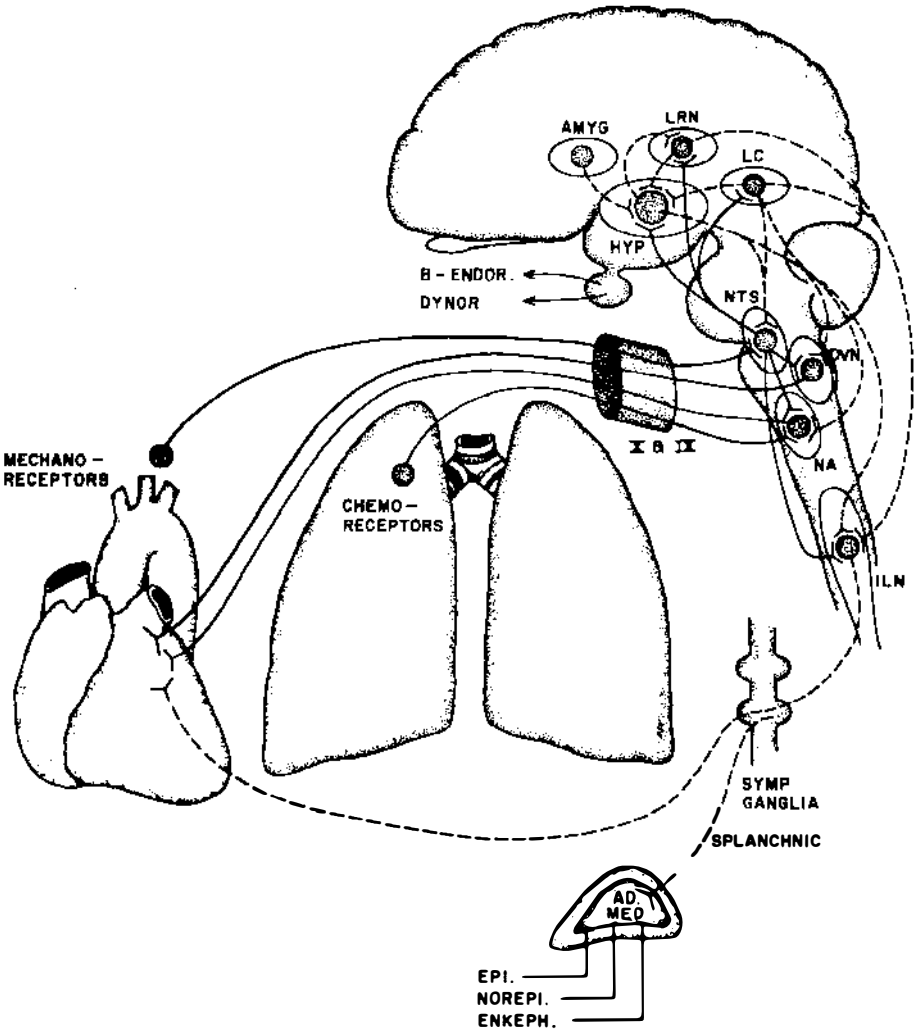


Figure 1 Central and peripheral autonomic pathways are represented in this simplified schematic of the neuronal circuitry that regulates cardiovascular responses. Solid lines generally represent parasympathetic innervations; dashed lines indicate pathways with greater direct relevance to sympathetic integration. The hypothalamus alone contains at least nine different nuclei with different anatomical and functional relationships among each other and with other autonomic nuclei. For a complete reference, see (26).

Abbreviations

AMYG Amygdala	NTS Nucleus tractus solitarius
LRN Lateral reticular nucleus	DVN Dorsal vagal nucleus
LC Locus coeruleus	NA Nucleus ambiguus
HYP Hypothalamus (many nuclei)	X Vagus nerve
ILN Intermediolateral nucleus	IX Ninth cranial nerve
β-ENDOR Beta endorphin	DYNOR Dynorphin
EPI Epinephrine	NOREPI Norepinephrine
ENKEPH Enkephalin	AD MED Adrenal medulla

(NA) and dorsal vagal nucleus (DVN). The parasympathetic vagal efferent component of the baroreflex arc originates from the NA, and this may be the primary site for integration of reflex bradycardia. The DVN also transmits efferent information via the vagus, possibly mediating both inotropic and chronotropic effects at the heart. Together, the NTS, NA, and DVN constitute the major parasympathetic components of the brainstem that modulate baroreflexes, chemoreflexes, and general parasympathetic outflow (25, 26).

The sympathetic pathways that are involved in autonomic effects upon the heart, vasculature, and adrenal medulla also receive information relayed from the periphery by the NTS. A major site for the integration of sympathetic outflow is the hypothalamus (Figure 1). This critical brain region is central to the orchestration of endocrine, somatic, autonomic, and emotional states, all of which have direct bearing upon the cardiovascular system.

The hypothalamus has reciprocal innervation with brainstem parasympathetic nuclei. It not only receives information from the NTS, but it projects information to the NTS, NA, and DVN. The efferent sympathetic outflow from the hypothalamus extends to the intermediolateral nucleus (ILN) of the spinal cord. In fact, the ILN is the primary final common pathway for preganglionic sympathetic outflow (26). It also receives projections directly from the NTS and other structures of the brain such as the locus coeruleus and reticular areas (Figure 1).

This much-simplified picture of parasympathetic and sympathetic networks is not intended to provide a complete framework for referencing opiate-autonomic interactions in the brain and periphery. For example, at least nine different hypothalamic nuclei are involved; limbic, cortical, and cerebellar nuclei (28) may also exert strong cardiovascular influences involving opiate systems (26, 27). Parasympathetic outflow is primarily relayed to peripheral end organs via the nodose ganglia, while sympathetic ganglia (e.g. cervical, mesenteric, etc.) relay sympathetic outflow to peripheral effector sites. Species differences in the details of autonomic integration must also be considered. However, even based upon this simplified schematic, it should be obvious that manipulating any one component or injecting opiates into any single nucleus will have relatively unpredictable consequences. Until a greater morphological understanding of the full network becomes available, pharmacological evaluation of these autonomic interactions will remain most difficult to interpret.

Autonomic Loci of Endogenous Opiates and Their Receptors

Enkephalin-containing neurons have been identified throughout the brain through the use of immunohistochemical procedures (24, 29–32). In the caudal medulla, enkephalin-positive material is found in the NTS, NA, and

DVN. These neurons are characterized by short projections, suggesting an interneuronal role. Opiate receptors are also similarly distributed in the brainstem near the terminals of enkephalin-containing neurons. In fact, the region around the area postrema near the obex is rich in opiate receptors (33); this autonomic locus is critical to cardiovascular control since all afferent baroreceptor fibers pass through this region (27).

By contrast with the widely distributed enkephalin-positive neurons, the cell bodies of β-endorphin-positive neurons are largely restricted to the arcuate nucleus of the hypothalamus, with long axonal projections extending to the NTS as well as reticular, midbrain, and limbic regions. Thus, as with enkephalins, the anatomical distribution of β-endorphin-containing neurons is consonant with actions that may affect circulatory function (34). Additionally, these neurons are immunoreactive for adrenocorticotropic hormone (ACTH) and β-lipotropic hormone (βLPH), two peptides that share the common proopiocortin precursor with β-endorphin (35).

The peripheral nervous system and endocrine organs also contain opioid peptides. Enkephalins have been demonstrated in the superior cervical ganglia as well as the inferior and superior mesenteric ganglia (36, 37). In the adrenal gland, the chromaffin cells of the medulla contain both enkephalins and catecholamines, and stimulation of the splanchnic nerve results in their concomitant release in a constant molar ratio (38). Thus, enkephalins in the circulation appear to derive predominantly from the adrenal medulla. By contrast, β-endorphin in the circulation originates from the anterior and intermediate lobes of the pituitary gland (Figure 1).

The localization of opiate receptors in the periphery is less well resolved than in the CNS. Young and colleagues (33) have demonstrated that the vagus nerve contains opiate receptors that are transported along axons from cell bodies in the nodose ganglia to the brain as well as to the periphery. Pharmacologic evidence (25) suggests that pulmonary "J" receptors in the lung may contain opiate binding sites. Vascular beds may also contain opiate receptors (39); however, specific vascular binding of opiates has not been reported. Although a small amount of opiate binding has been demonstrated in the heart, studies with whole heart homogenates have revealed that these binding sites were not stereospecific (40). Indeed, this is an area where more work is needed. The massive ratio of muscle to impulse-propagating tissue in the whole heart may have diluted the possibility of demonstrating the existence of specific opiate receptors. Instead of whole heart binding, it may be more relevant to analyze binding characteristics in specific tissues derived from nodal regions or the bundles of His.

What about enkephalins and β-endorphin released peripherally? Can they function as hormones to alter peripheral or central cardiovascular activity? At least two possibilities allow for such a hypothesis. First of all,

although little evidence is available, direct peripheral opiate effects upon the heart or vascular beds cannot be excluded (see above). However, such studies must rule out indirect effects of opiates through secondary histamine release. Secondly, direct actions upon autonomic nuclei in the brain are also possible. Although the blood-brain barrier may be expected to exclude these large, polar molecules from general access to the central nervous system, we have speculated that circulating β-endorphin (and possibly enkephalins) may gain access to autonomic centers in the brain via circumventricular sites which lack a blood-brain barrier, such as the area postrema and subfornical region (7, 41, 42). As noted above, the area postrema is densely populated with opiate receptors and critically located at brainstem autonomic sites that modulate cardiorespiratory function; the subfornical region above the hypothalamus could allow for diffusional access of opiates to hypothalamic centers. Such interactions would also be pertinent to the understanding of central cardiovascular responses to other neuropeptides (43).

Adrenal enkephalins have a far shorter half-life than β-endorphin, which may limit adequate concentrations from reaching brain centers to affect cardiovascular responses. However, circulatory anatomy is such that adrenal venous effluents (which would be rich in adrenal enkephalins following sympathetic activation) go directly to the right heart via the vena cava and then to the lung (Figure 2). Indeed, it has been shown that blood from the adrenal vein is at least tenfold more enriched with enkephalin than blood obtained from the femoral vein (20). Perhaps the heart and/or pulmonary chemoreceptors are the anatomical targets mediating cardiovascular responses following adrenal-medullary enkephalin release. Moreover, since the lung is rich in peptidase activity, which would rapidly degrade enkephalin following actions at such sites as pulmonary "J" receptors (Figure 2), the actions of enkephalin would be limited to that organ.

Taken together, it is plausible that endogenous opioid peptides released as peripheral neurohormones may alter the autonomic regulation of cardiovascular function by actions at peripheral opiate receptors, which serve to transmit afferent information to autonomic nuclei in the brain, or by gaining direct access to brain receptors located near circumventricular regions. In fact, recent work by Hanbauer and colleagues (44) has provided important evidence that adrenal enkephalins can function as hormonal modulators of circulatory variables. Those studies demonstrated that, in the anesthetized dog depleted of adrenal catecholamines by reserpine pretreatment, stimulation of the splanchnic nerve resulted in the release of adrenal enkephalins and a concomitant depression of heart rate and arterial pressure that were naloxone-reversible. From the work of Eiden & Ruth (45), it is suggested that circulating enkephalins can also modulate the effects of other cardio-

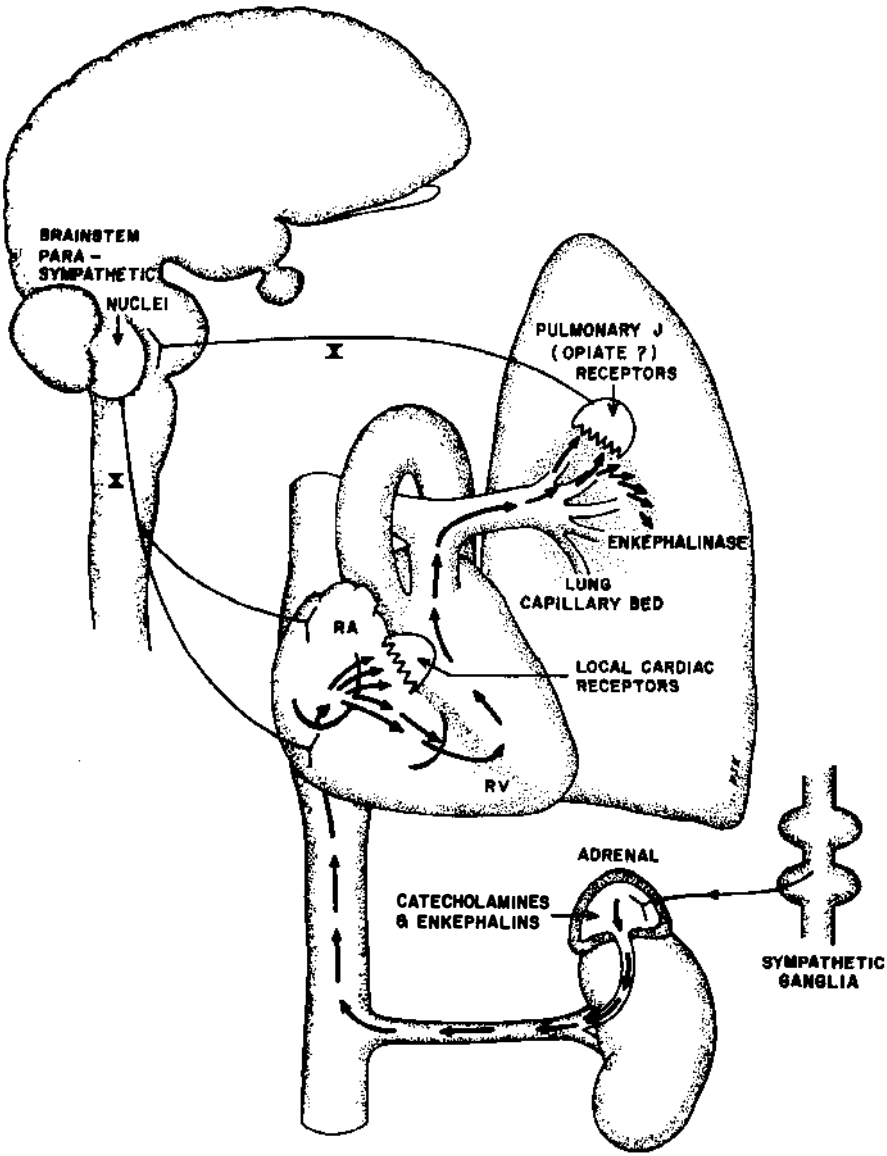


Figure 2 A hypothetical model depicting the functional proximity of the adrenal venous effluent relative to potential sites of direct hormonal actions at receptors in the heart or lungs. The close vascular access between the adrenal gland, heart, and lungs may provide for functional concentrations of adrenal enkephalins (see arrows) at these possible target sites. Within the lung, duration of enkephalin actions at pulmonary J receptors would be limited by the high concentrations of peptidases localized in that organ. Direct actions of catecholamines in the heart may be modulated by enkephalins (45), and enkephalins may be the endogenous ligands that activate pulmonary J receptors (25, 82).

tonic substances. Specifically, using isolated rat atria, these investigators demonstrated that, although various opioid peptides had no direct effect at concentrations up to 10^{-5}M, as little as 10^{-7}M enkephalin significantly antagonized the chronotropic effects of norepinephrine.

PHARMACOLOGICAL EFFECTS OF OPIATE PEPTIDES UPON CIRCULATORY FUNCTION

Effects of Central Nervous System Administration

The cardiovascular effects of pharmacologically administered opioid peptides, like the alkaloid opiates (7), are complex and dependent upon species, doses, route and site of administration, presence or absence of anesthetics, and other interactions. With consideration to the above variables, opioid peptides can produce tachycardia or bradycardia as well as hypertension or hypotension. The following section outlines evidence for the influence of these factors upon cardiovascular responses to injected opioid peptides.

Although the direct effect of opioid substances upon secondary neuronal systems is usually inhibitory (46), another glance at Figure 1 reveals the complex networks that may be involved in the ultimate expression of opioid effects upon hemodynamics. For example, if injected opiates inhibit an inhibitory neuron, excitation of the next system may occur. Such networks within and among autonomic nuclei, which can depress (parasympathetic) or stimulate (sympathetic) circulatory function, further complicate prediction of the ultimate hemodynamic responses to opiates. Nonetheless, demonstration of a cardiovascular response to administered opiates will help define the potential importance of the endogenous opiate system in circulatory function.

Florez & Mediavilla (11) were among the first to report on the cardiovascular effects of opiate peptides. They demonstrated that application of Met-enkephalin to the ventral surface of the brainstem produced a naloxone-reversible hypotension and bradycardia. Laubie (13) found that β-endorphin injected into the cisterna magna of chloralose-anesthetized dogs produced an initial, transient elevation of heart rate and arterial pressure, followed by delayed bradycardia and hypotension. Furthermore, these investigators demonstrated that the NA in the brainstem was a selective and highly sensitive area for the vagal bradycardia produced by Met-enkephalinamide (46a). Using anesthetized cats, Feldberg & Wei (12) obtained pressor and tachycardic effects upon injection of enkephalin molecules into the lateral ventricles, whereas cisternal injections favored the opposite results.

Bolme and colleagues (24, 47) defined the responses to various opioid peptides on a continuum from those that generally resulted in a pressor effect (e.g. Leu-enkephalin) to those that produced a depressor effect follow-

ing intracisternal injection in anesthetized rats (e.g. β-endorphin). They reported that the hypertensive responses to centrally injected opioid peptides were generally resistant to naloxone blockade, whereas the hypotensive effects were readily blocked by naloxone. They speculated that different subtypes of opiate receptors mediate these differential opiate effects. Simon et al (48), working with conscious rats, observed that the pressor response to centrally administered enkephalin was blocked by propranolol and hence presumably catecholamine-mediated. In support of that suggestion, van Loon et al (49) have observed that intracisternal β-endorphin injections produced increases in circulating concentrations of epinephrine, norepinephrine, and dopamine.

Recent work by Yukimura et al (50) has demonstrated that d-Ala^2-Met^5 enkephalinamide (DAME) injected intracerebroventricularly in conscious rats and cats produced a pressor response. Yukimura et al observed that, in contrast with the findings of Bolme et al (47), this pressor response to DAME was completely blocked by naloxone. They further reported that the depressor response to DAME in anesthetized animals (51) was resistant to naloxone blockade but readily antagonized by diprenorphine, an opiate antagonist with greater delta receptor selectivity (52). At further variance with the above results, Owen and associates (53) reported that DAME injections into the third ventricle of conscious monkeys produced a pronounced depression of arterial pressure and heart rate that was readily antagonized by subsequent naloxone administration. Species differences and the state of consciousness of the experimental animals are important variables in these disparate studies (vide infra).

In the studies summarized above, opioid peptides were applied directly to the surface of the brain or injected into ventricular spaces. Even within a given ventricular region in a single species, different responses can be evoked by injected opioid peptides. For example, Feuerstein & Faden (54) have recently demonstrated that the same opiate agonist injected into two periventricular hypothalamic nuclei (separated by less than one micron in the rat) produced opposite effects upon arterial pressure.

The state of consciousness of experimental animals has also been shown to be an important variable in interpreting the pharmacological responses to injected opiates. Delbarre et al (55) first reported that anesthetics could block the pressor response to centrally injected opioid peptides. More recently, Sander et al (56) and Yukimura et al (52) have systematically studied the alteration of cardiovascular responses following opiate injections in anesthetized and unanesthetized animals. In general, it has been observed that anesthetics blunt the pressor response to injected opiates and enhance their depressant effects.

A series of studies were recently conducted in our laboratories to evaluate the pharmacological effects of prototype mu and delta opiate receptor li-

gands relative to their cardiorespiratory actions at third or fourth ventricular sites in anesthetized and unanesthetized rats (57). Another objective of these experiments was to evaluate the influence of these variables in order to allow for an integrated assessment of their relative importance in the expression of cardiovascular responses to injected opiates.

Morphine and β-casomorphin 1-4 (morphiceptin) were chosen as prototypic mu agonists (58–60), and d-Ala^2-d-Leu^5-enkephalin (DADLE) was used as a prototype delta ligand (61). Doses used in these studies represented the minimum amount of agonist required to produce a 10 mmHg change in arterial pressure. As seen in Figure 3, in conscious rats DADLE evoked a pressor response following fourth ventricular injection, while morphiceptin produced a pressor response when injected into the third ventricle. The presence of light anesthesia (30 mg/kg pentobarbital) eliminated the pressor response to these opioid peptides and enhanced depressant responses (Figure 3).

In more thorough additional studies, it was demonstrated that the predominant effect of DADLE after third ventricular injection in anesthetized rats was to produce a severe hypotension accompanied by a decrease in pulse pressure, possibly indicating an action at hypothalamic delta opioid receptors in decreasing sympathetic outflow. By contrast, morphine exerted pronounced bradycardic effects following fourth ventricular administration, suggesting an action at brainstem mu opioid receptors that influence vagal-parasympathetic activity (57). Both agonists resulted in a depression of respiratory rates following fourth ventricular injection, indicating the potential importance of this site in mediating the respiratory effects of opiates. These cardiorespiratory responses to opiates in lightly anesthetized rats may provide insights into the anatomical locations and opiate receptor subtypes involved in mediating these autonomic actions of opioids. Alternatively, because of the complexities of the system mentioned above, it is likely that these studies will only confirm that pharmacological evaluations of opiate agonist effects are complicated by variables such as injection sites, opiate receptor subtypes, and the state of consciousness of the experimental animals.

Effects of Peripherally Injected Opioid Peptides

As with centrally injected opioid peptides, peripheral injection of these substances can produce increases or decreases in cardiovascular variables. Unlike centrally administered opioid peptides, however, the possibility that peripheral opiate injections can produce secondary cardiovascular responses due to histamine release from mast cells in certain species must be considered. Once again, anesthetics can dramatically alter the direction of change in arterial pressure and heart rate. Additionally, the intravenous injection of these substances often results in biphasic responses, with initial

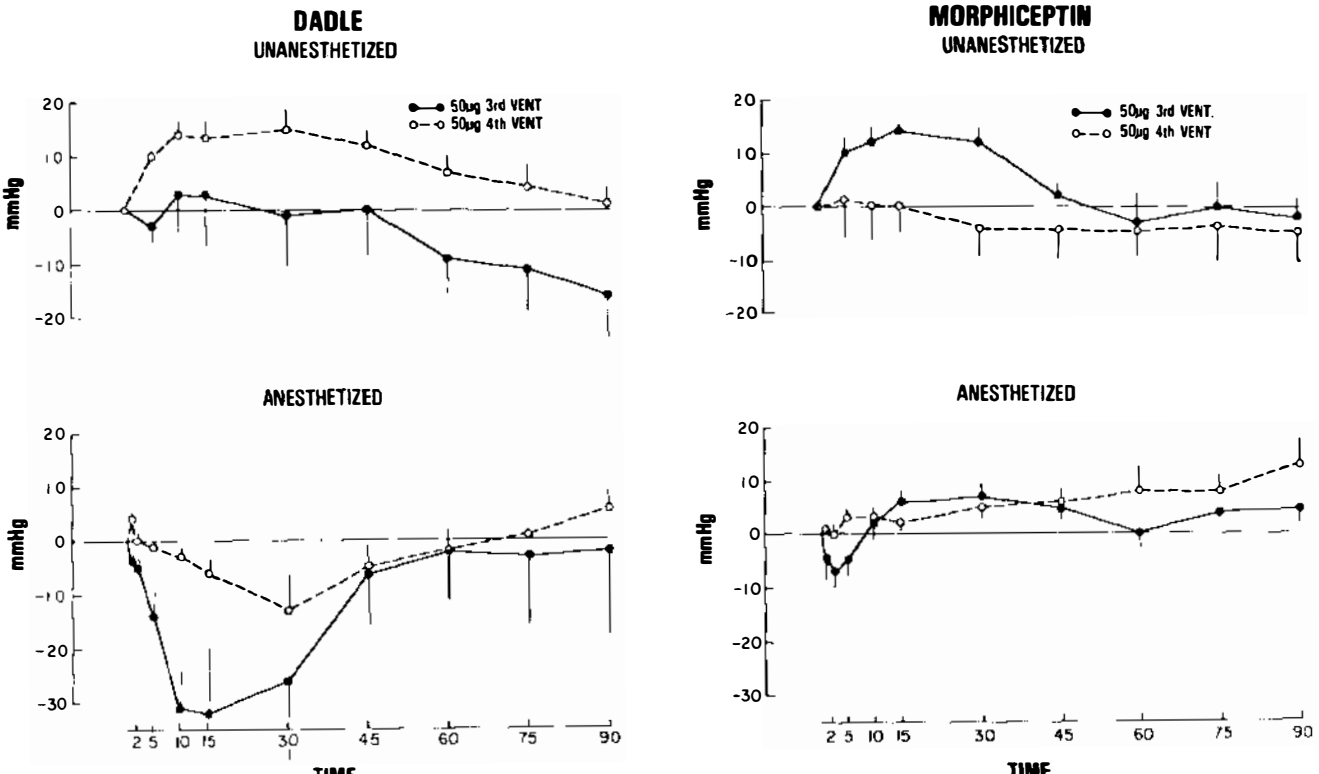


Figure 3 Comparison of the effects of relatively selective opioid agonists at different injection sites in unanesthetized and anesthetized rats; data for change in mean arterial pressures are compared. β-casomorphin (1–4) amide (morphiceptin; 96 nM) was used as the μ-agonist, and DADLE (88 nM) was used as the δ-agonist; anesthetized rats were given 30 mg/kg pentobarbital. Doses were chosen as the minimum amount of peptide to produce at least a 10 mmHg change in arterial pressure. At least 6 rats were used per data point; vertical bars are SEM. These data point to the confusing effects of these variables upon cardiovascular endpoints. (See text for details).

transient effects lasting less than a minute followed by delayed responses of much longer duration (5, 14, 15, 62).

Feldberg & Wei (12) observed a brief depressor effect of DADLE following intravenous (iv) injection at 30 μg/kg in anesthetized cats. Moore & Dowling (62) demonstrated potent, biphasic responses to intravenous enkephalins in anesthetized cats, with initial pressor responses followed by delayed hypotension; both histamine and catecholamine involvement were shown to influence the pressor response to Leu-enkephalin. Wei et al (15) defined the bradycardic responses to a variety of iv opioid peptides in anesthetized rats and demonstrated the mediation of this chronotropic response by vagal mechanisms. Sapru et al (25) also found bradycardia and hypotension following opioid peptide administration into the right atrium of decerebrate rats.

Simon et al (48) observed progressive increases in arterial pressure following intravenous injections of Met- and Leu-enkephalin in conscious rats. Met-enkephalin was more potent in inducing increases in arterial pressure, possibly owing to its additional selectivity over Leu-enkephalin in increasing heart rate. Schaz and associates (63) reported that intravenous Leu-enkephalin produced a greater pressor response in spontaneously hypertensive rats than in normotensive control animals. Evidence supporting a peripheral site of action for these actions of Leu-enkephalin was obtained by studies demonstrating that intravenous, but not intraventricular, naloxone blocked the pressor response to intravenous opioid-peptide challenge (63).

Recent work by Sander et al (64) has defined the cardiopulmonary effects of enkephalins in the conscious dog. They found that the enkephalin molecules produced a dose-dependent, albeit transient, increase in heart rate, arterial pressure, and respiratory rates in this model.

Not only are the direction of heart rate and arterial pressure changes altered, but the potency of opiate ligands is dramatically enhanced in the unconscious organism. Lemaire et al (14) successfully demonstrated a depressor response to intravenous β-endorphin at doses as low as 30–150 μg/kg in anesthetized rats. By contrast, our experience with conscious rats revealed that intravenous doses as high as 3–10 mg/kg β-endorphin (about 100-fold higher) were required for transitory bradycardia and hypotension [(65), Holaday and Faden, unpublished]. In conscious monkeys, however, 0.7 to 2.8 mg/kg β-endorphin administered intravenously resulted in a transient tachycardia followed by bradycardia. Arterial pressure was significantly reduced for over an hour. These doses also diminished scheduled behavioral responses in monkeys. Nonetheless, in both conscious rat and monkey studies, on a molar basis intravenous β-endorphin was two to three times more potent than morphine in evoking cardiodepressant and behavioral effects (65, 66).

Multiple Opiate Receptors and Cardiovascular Responses to IV Morphine

The use of relatively selective opiate agonist ligands provides one approach to an understanding of the importance of specific opioid receptor subtypes in cardiorespiratory response to opiate substances (vide supra). We have conducted a series of studies designed to evaluate this issue from yet another perspective. Recently, opiate antagonists with relatively selective effects for specific opiate receptors have become available (67–69). The following describes the cardiorespiratory responses to morphine in animals treated with such antagonist compounds.

In collaborative studies (70), rats were initially pretreated with naloxazone (50 mg/kg iv), an irreversible opiate antagonist that has been shown by Pasternak et al (67) to have selectivity for high affinity mu_1 binding sites (Holaday et al). Twenty-four hours later, these conscious rats (as well as saline-pretreated controls) were challenged with 100 mg/kg morphine sulfate (iv); behavioral, cardiovascular, colonic temperature, and respiratory responses were compared. Following morphine challenge, saline-pretreated rats demonstrated maximal increases in tail flick latencies and catalepsy that remained elevated for 24 h; colonic temperatures fell by 2½ degrees centigrade over the first two hours (70). By contrast, this dose of naloxazone completely blocked the cataleptic effects of this enormous morphine dosage; only transient increases in tail flick latencies and body temperature resulted.

Naloxazone pretreatment significantly attenuated the morphine-induced hypotension and hypoventilation. Interestingly, naloxazone-treated rats experienced a slight increase in pulse pressure in response to morphine challenge, and morphine-induced bradycardia was poorly antagonized by naloxazone. Despite these dramatic differences in cardiorespiratory, temperature, and behavioral response between naloxazone-pretreated and saline control rats, this dose of morphine was 20% lethal in both groups of animals (70). This observation is consistent with earlier results demonstrating a lack of effect of naloxazone on morphine-induced mortality (67).

More recently, the effects of various morphine doses were evaluated following treatment with β-funaltrexamine (β-FNA), a long lasting, mu-selective opiate antagonist described by Takemori et al (68). Additionally, morphine responses were compared in rats treated with M154,129, a diallyl-Tyr^1-enkephalin derivative with delta antagonist actions (69). Comparison of the alteration in analgesic responses to morphine in βFNA- and M154,129-pretreated rats revealed that βFNA significantly antagonized the increase in nociceptive latencies produced by morphine (at doses within the range of cardiorespiratory responses), whereas M154,129 failed to do so (71,

72). Since the antinociceptive effects of morphine are presumably mu-mediated, this observation confirms that βFNA is a mu antagonist and that M154,129 is relatively devoid of mu antagonist activity.

βFNA pretreatment resulted in an 8-fold rightward shift of the bradycardic dose response curve to morphine challenge (71). In conscious rats, doses of morphine in excess of 100 mg/kg iv were required before significant hypotension was produced; both βFNA- and M154,129-treated rats were more resistant to this effect (71). As determined by blood gas measurements, morphine-induced depression of respiration was about equally antagonized by both substances [4–5-fold shift; (72), Ward and Holaday, in preparation].

It must be pointed out that the "selective" opiate agonist or antagonist molecules studied may not have *absolute* selectivity for one opioid receptor subtype over another. For example, morphine has a greater selectivity for action at mu opioid receptors, although crosstalk with other receptor subtypes does occur at higher morphine dosages. Nonetheless, within the constraints of these limitations, the results of the antagonist data reviewed above indicate that mu receptor binding is critical to the decrease in heart rate produced by morphine, whereas both delta and mu actions may be involved in the hypotensive and respiratory depressant actions of this ligand. These results are compatible with the conclusions drawn from opiate agonist studies using morphine and DADLE (57).

Baroreceptor Reflexes and Opioid Agonists

The depressant effects of morphine on baroreceptor reflexes in animals and man have been known for many centuries. In 1895, Hill (1) reported that opiates induced severe orthostatic hypotension in dogs. Drew and colleagues (73) evaluated the effects of morphine on orthostatic responses in humans over 35 years ago. As predicted from these responses to alkaloid opiates, injections of opioid peptides also blunt baroreceptor reflexes. In fact, among the various cardiovascular responses to injected opiates, their effect in inhibiting baroreceptor reflexes is among the most clear cut.

Schaz and associates (63) infused angiotensin II and found that d-Ala^2-Met^5 enkephalin (DAME) attenuated the compensatory decrease in heart rate following infusion of this pressor substance. Moreover, heart rate did not increase following the pressor response to DAME itself, thus indicating an inhibition of baroreceptor responses. Petty & Reid (74) used phenylephrine to increase, and nitroprusside to decrease arterial pressure in order to evaluate both bradycardic and tachycardic responses to baroreceptor activation. Enkephalin analogs attenuated reflex responses, and naloxone resulted in their exaggeration (74).

Further studies by Freye & Arndt (75), Montastruc et al (76), and Sander et al (64) have confirmed that injected opiate peptides blunt baroreflex responses in a number of species. The physiological involvement of endogenously released opiates in baroreceptor reflexes has been shown by us and others (vide infra). Additionally, the collective evidence reviewed above shows that baroreceptor systems appear to be exquisitely opiate-sensitive; following injection of doses of opiates too low to cause direct cardiovascular effects, baroreceptor reflexes may be significantly inhibited.

Cardiovascular Effects of Nonopioid Peptide Metabolites of Endogenous Opiates

As reviewed above, the pressor response to injected enkephalin peptides has been reported to be refractory to antagonism by naloxone (24, 47). This could mean that different subpopulations of opioid receptors are involved that are less sensitive to the more mu-selective antagonist action of naloxone. However, high enough doses of naloxone were administered to overcome differential receptor affinities by mass action, making this explanation less plausible. Alternatively, it could be speculated that part of the hypertensive effects of injected enkephalin molecules were not due to actions at opiate receptors, but were mediated by nonopiate mechanisms activated by enkephalins or their metabolites.

During the isolation and characterization of the precursor molecule for adrenal medullary enkephalins, Lewis et al (77) defined a novel opioid heptapeptide, Met-enkephalin Arg^6-Phe^7. The last four amino acids of this molecule, namely Phe-Met-Arg-Phe, are the same as in molluscan cardioexcitatory neuropeptide as described by Price & Greenberg (78). In fact, Greenberg et al (79) classified the opioid heptapeptide described above as a potent analog of the molluscan cardioexcitatory neuropeptide.

Is the Phe-Met-Arg-Phe cardioactive tetrapeptide generated in vivo from the opioid peptide Met-enkephalin Arg^6-Phe^7? Marks et al (80, 81) reported that a metalloendopeptidase present in brain and kidney could generate the cardioactive tetrapeptide from Met-enkephalin Arg^6-Phe^7. Moreover, these investigators demonstrated that, although rat heart homogenates did not generate the cardioactive tetrapeptide, Met-enkephalin was cleaved from Met-enkephalin Arg^6-Phe^7 to yield Arg-Phe.

Recent work by Wei and associates (82) has shown that Arg-Phe produces increases in cardiovascular function that are not naloxone-sensitive. Thus, the pressor response to injected opiate peptides such as Met-enkephalin Arg^6-Phe^7 may be a consequence of nonopiate metabolites of that peptide such as Phe-Met-Arg-Phe or Arg-Phe. Further, as Marks et al (81) have shown that angiotensin-converting enzyme (ACE) inhibitors (e.g. captopril) prevent the cleavage of Arg-Phe from the precursor, it is suggested

that the antihypertensive effects of such ACE inhibitors may involve this action in addition to their well-known effects in the prevention of angiotensin II formation.

Other nonopiate actions of enkephalin-derived molecules upon arterial pressure have been reported by Sander & Giles (83). Using a des-Tyr analog of Leu-enkephalin (des-Tyr^1-d-Ala^2-Leu^5 enkephalinamide), they observed a pressor response following intravenous injection of this tetrapeptide enkephalin analog in conscious dogs. This vasopressor action was not antagonized by naloxone. Thus, the collective results reported above indicate that the C-terminal fragments of enkephalins and the enkephalin heptapeptide may be partially responsible for pressor effects of these molecules and that this action is unrelated to opioid activity.

PHARMACOLOGICAL PERSPECTIVES AND CONCLUSIONS

Review of the literature summarized above reinforces the problems of interpreting pharmacological studies conducted with different injection sites in different species using various opioid ligands. The fact that different opioid ligands produce qualitatively different cardiovascular effects indicates that more than one opioid receptor subtype may be involved, possibly at different anatomical sites. Moreover, low doses of a given ligand may produce a pressor response, whereas higher doses often result in the opposite effect. Conclusions regarding cardiorespiratory responses to administered opioids are further complicated by quantitative and qualitative differences resulting from the state of consciousness of the experimental animal. Opioid effects upon secondary factors, such as peripheral catecholamines, histamine release, or central neurotransmitters must also be considered.

Perhaps it is without solace to point out that the opiate system is not uniquely complex with regard to cardiovascular pharmacology and physiology. Anesthetics blunt or abolish the pressor effects of centrally injected angiotensin II (43). Bradykinin, an endogenous nonapeptide found in the circulation, is hypotensive upon peripheral injection, yet it results in an increased arterial pressure upon injection into brain ventricles (23). If the undecapeptide substance P is dissolved in saline as opposed to artificial cerebrospinal fluid, the pressor response following ventricular injection is far greater (24). Injected intravenously, clonidine (the antihypertensive α-agonist drug) results in an initial pressor response followed by a protracted depressor response. Microinjection of clonidine into the NTS or anterior hypothalamus lowers arterial pressure, whereas posterior hypothalamic clonidine injections produce pressor responses (26). From a more physiologic perspective, electrical stimulation of the anterior hypothalamus de-

presses heart rate and blood pressure, whereas posterior hypothalamic stimulation has the opposite effect; these responses are also modified by anesthetics (26).

Thus, given the complexity of anatomical pathways by which autonomic responses can be mediated (Figure 1), the complex cardiovascular pharmacology of opiate ligands is not unexpected. Although some contradictions exist, it may be possible to derive a few broad generalizations from the pharmacological studies described above.

The existence of multiple opiate receptors has been well established in vitro (84–86) and in vivo using spinally transected dogs (58). However, autonomic responses following activation of specific opiate receptor subpopulations have been less well defined in intact animals. We have shown that prototype mu and delta opiate ligands can produce apparently selective responses depending upon the site of ventricular injection (57). Nonetheless, two important caveats must be considered in interpreting in vivo studies designed to address this issue: 1. Similar to the classic alpha and beta adrenergic systems, it is probable that the relative selectivity for one receptor over another for most ligands will *not* be absolute, i.e. mu agonist may have some delta or kappa effects, etc. 2. This lack of absolute receptor specificity requires that full dose-response data be compared between a known effect (e.g. antinociception) relative to doses required to elicit changes in arterial pressure or heart rate, for example.

Although the work of several authors as summarized above may suggest that different opiate receptor subtypes produce different autonomic effects, thorough quantitative studies using best available ligands are still pending. Nonetheless, a testable working hypothesis is proposed that the parasympathetic effects of injected opiates are more directly "mu"-mediated via actions at brainstem sites (71). Like alkaloid opiates, opioid peptides that also have prominent analgesic effects and more "mu"-like actions in vitro appear to induce bradycardia (15) and hypotension through brainstem actions (57). Evaluation of the data presented by Wei and colleagues (15) reveals that the relative potencies for bradycardic effects of opiates in anesthetized rats roughly parallels the relative analgesic potencies known for these substances. Additionally, vagotomy and atropine were shown to block opiate bradycardia in their studies (15) as well as those by Fennessy & Rattray (5), thus pointing to the involvement of vagal-parasympathetic systems in the negative chronotropic effects produced by opiate analgesics.

By contrast, opioid peptides (e.g. enkephalins) that have minimal analgesic potency tend to produce opposite cardiovascular effects (vide supra). It is possible that injection of these substances, such as Leu-enkephalin or DADLE, results in prominent actions upon "delta" receptors, which can stimulate central sympathetic outflow (hypothalamic?), thus elevating

arterial pressure through sympathetic discharge and/or adrenal medullary secretion. This would be consistent with the observations of van Loon and colleagues (47), who report increased plasma catecholamine concentrations after central opioid peptide injections, as well as other studies that demonstrate that adrenergic antagonists block pressor responses to injected opioids (27, 48, 62).

According to Bolme et al (47), pressor responses following intracisternal enkephalin or β-endorphin injections in anesthetized rats were resistant to blockade by naloxone, whereas depressor responses were almost entirely blocked by this same naloxone dosage. Owing to the approximate tenfold lower selectivity of naloxone for delta as opposed to mu binding sites, this observation would be more consistent with the opioid pressor responses being delta- and depressor responses mu-mediated. Alternatively, pressor responses to opiate heptapeptides may also involve nonopiate actions of their metabolites (vide supra).

At variance with the above, our investigations with DADLE and morphine in anesthetized rats revealed that DADLE had pronounced depressor effects following third ventricular injection in lightly anesthetized rats. Presumably, hypothalamic sites were bathed by this delta ligand. However, these rats were anesthetized with pentobarbital, a factor that profoundly alters the direction as well as potency of cardiovascular responses to injected opiates.

The mechanism(s) by which analgesia alters opiate effects upon cardiovascular endpoints is also subject to speculation. Lang and colleagues (22) suggest that anesthesia has a specific effect to inhibit mu receptors, whereas presumed delta-receptor-mediated actions were reportedly unaffected by anesthetics. Although their data do not conclusively support specific mu and delta-mediated responses to anesthetics, it is possible that differences at the level of opioid receptor subtypes are involved.

General CNS-depressant effects of anesthetics may also be relevant; Korner (27) reviews literature suggesting that the diencephalon is depressed by anesthetics. Since diencephalic-hypothalamic sites may be responsible for opiate pressor responses, their inhibition by analgesics could decrease sympathetic tone and thus facilitate parasympathetic inhibitory actions. This simplistic reasoning must be contrasted with the known vagolytic actions of anesthetics, which would tend to produce the opposite result.

From a more general view, it is proposed that the degree of "arousal" may be the most important factor in modifying opiate responses, not anesthesia per se. For example, opiates are classified as analgesics since pain relief is obtained without loss of consciousness. However, at analgesic doses in animals, behavioral as well as autonomic endpoints such as temperature and respiratory function are certainly depressed by opiates. As noted above,

opiate substances with a greater analgesic effect also tend to have a more prominent depressor effect upon heart rate and arterial pressure.

It appears possible, therefore, that the depressant effects of opiates upon cardiovascular variables are interdependent with the potency of such substances in depressing pain, body temperature, respiratory function, etc. A logical site for such integrated depressant effects may include the hypothalamus since this region is critical to the coordinated regulation of emotional, somatic, autonomic, and endocrine systems. Thus, in addition to hypothalamic set points for temperature regulation, central "thermostats" may also be involved in the integrated homeostasis of mood, respiration, and cardiovascular function. Such a hypothesis becomes particularly attractive when compared to the parallel directional shifts in body temperature that are also modified by specifics of ligand, dose, and time (for review see 7).

Despite the apparent sophistication of experiments regarding the anatomy and acute effects of injected opiates, the fundamentals of opiate pharmacology have been largely ignored. Few data are available regarding tolerance to opiate-cardiovascular effects or the circulatory consequences of withdrawal following physical dependence. Additional research into peripheral actions of opiates at the heart or on vascular beds is required. The availability of relatively selective agonists and antagonists for opioid specific receptor subtypes has provided some preliminary evidence that different cardiovascular responses are mediated by different receptors (15, 57, 71), although further work in this area is needed.

ENDOGENOUSLY ACTIVATED OPIATE SYSTEMS AND CARDIOVASCULAR FUNCTION

Anatomical and pharmacological data reviewed above demonstrate that endogenous opiate systems exist within neuronal networks and hormonal localizations consistent with their possible actions upon autonomic pathways involved in regulation of the heart and vasculature. However, injecting opiate agonists into veins, arteries, brain ventricles, or even discrete autonomic nuclei, addresses pharmacological responses that may have little bearing upon the potential role of endogenous opiate systems in altering cardiovascular activity. Compelling evidence that endogenous opiate systems help regulate cardiovascular function derives from the results of opiate antagonist injections in various physiologic and pathophysiologic states. Changes in hemodynamic variables following opiate antagonist administration suggest that endogenously activated opiate systems have been interrupted, thus preventing their actions in altering circulatory function.

The remainder of this review focuses upon the use of opiate antagonists that act at the receptor level (e.g. naloxone and related substances) as well as "physiologic" opiate antagonists that reverse selected opiate actions through their own receptor and effector systems (e.g. thyrotropin releasing hormone). These tools have provided evidence that endorphins are involved in the pathophysiology of shock, spinal injury, orthostatic hypotension, anesthetic hypotension, and possibly in essential hypertension as well as cerebral vascular disorders. These basic science findings indicate possible novel applications of opiate antagonists in the treatment of a variety of cardiovascular disease states.

The Function of Endogenous Opiates in Shock and Trauma

Earlier work showed that many of the signs and symptoms of opiate overdose strongly resemble those of circulatory shock. These include not only a compromised cardiovascular function, but also a lack of responsiveness to nociceptive stimuli, an alteration of endocrine systems, and a disruption of normal body temperature. Given these similarities and the knowledge that severe stress activates endogenous opiates (87), we proposed that endogenous opiates, released by the severe stress accompanying shock, would act like morphine and thereby contribute to the decrease in circulatory function that characterizes the shock syndrome. We initiated a series of studies to test the hypothesis that the blockade of opioid receptors by opiate antagonists such as naloxone should reverse the endogenous opiate component of shock hypoperfusion and improve cardiovascular function as well as survival (16–18).

ENDOTOXIC SHOCK Studies with gram-negative endotoxins have been widely used as models to assess pathophysiological responses and potential pharmacological tools in septic shock (88). In the initial experiments designed to evaluate a pathophysiologic role of endogenous opiates in circulatory shock, we studied endotoxemia in conscious rats (16). Following the intravenous administration of the lipopolysaccharide endotoxin derived from *E. coli,* mean arterial pressures fell precipitously within minutes to a level 25–30 mm Hg below control values. This hypotension was reversed, in a dose-dependent manner, by naloxone injections at doses between 0.1 and 10.0 mg/kg intravenously (89). Following these initial observations, we conducted additional studies to assess the possible involvement of opiate receptors in mediating the improved hemodynamic responses to naloxone in this model. The active (–) naloxone isomer improved blood pressure following endotoxic hypotension; the (+) isomer, which does not bind opiate receptors in vitro or in vivo, was without therapeutic effect. These data provided evidence for a stereospecific action of naloxone at opiate

receptors, probably by competitive antagonism of endogenous opiate peptides at the receptor level (89).

To evaluate species specificity, and in order to perform more extensive cardiovascular monitoring, collaborative studies were conducted with Reynolds, Gurll, Vargish, and Lechner to evaluate the therapeutic effects of naloxone in dogs subjected to endotoxic shock (90). Following the administration of endotoxin and its resultant depression of hemodynamic variables, a 2.0 mg/kg dose of naloxone significantly improved cardiac contractility, stroke volume, cardiac output, and mean arterial pressure (MAP) when compared to saline-injected controls. By contrast, naloxone had no significant effect upon venous pressure, pulmonary artery wedge pressure, heart rate, or calculated total peripheral vascular resistance. A critical finding from these studies was that naloxone treatment also dramatically improved survival.

At this juncture, it is important to note that endogenous opiate systems are normally quiescent until homeostatic processes are disrupted. At least with regard to circulatory function, naloxone injections into unanesthetized or anesthetized control animals that were not subjected to shock produced no significant alterations of cardiovascular variables (for review see 7). From this, we can conclude that naloxone has no pressor effects of its own. Instead, naloxone appears to exert a selective action in reversing an endogenous opiate-mediated depression of circulatory function.

Experiments in other laboratories have confirmed that naloxone exerts significant therapeutic effects in endotoxic shock in a number of animal species. Wright & Weller (91) observed that, in mice subjected to endotoxic shock, naloxone reversed the fall in body temperature and decreases in circulating white blood cells and platelets. Raymond et al (92) found that naloxone attenuated the hypotension, hemoconcentration, acidosis, and hypoglycemia and also improved survival in dogs subjected to endotoxic shock. Weld and colleagues (93) demonstrated that endotoxic shock in horses responds to the therapeutic effects of naloxone. Naloxone also improves hemodynamic function in live *E. coli* sepsis; Gahhos et al (94) have reported that naloxone improved cardiovascular function in pigs injected with a suspension of this organism. Most recently, Gurll and associates (95) showed that monkeys experienced an improvement in cardiovascular variables and survival when naloxone was administered following the induction of endotoxic shock.

Mechanisms and sites of naloxone's therapeutic effects in endotoxic shock

The precise mechanisms and sites involved in mediating the therapeutic effects of naloxone in experimental models of sepsis have been explored in a number of laboratories. From studies demonstrating the stereospecificity

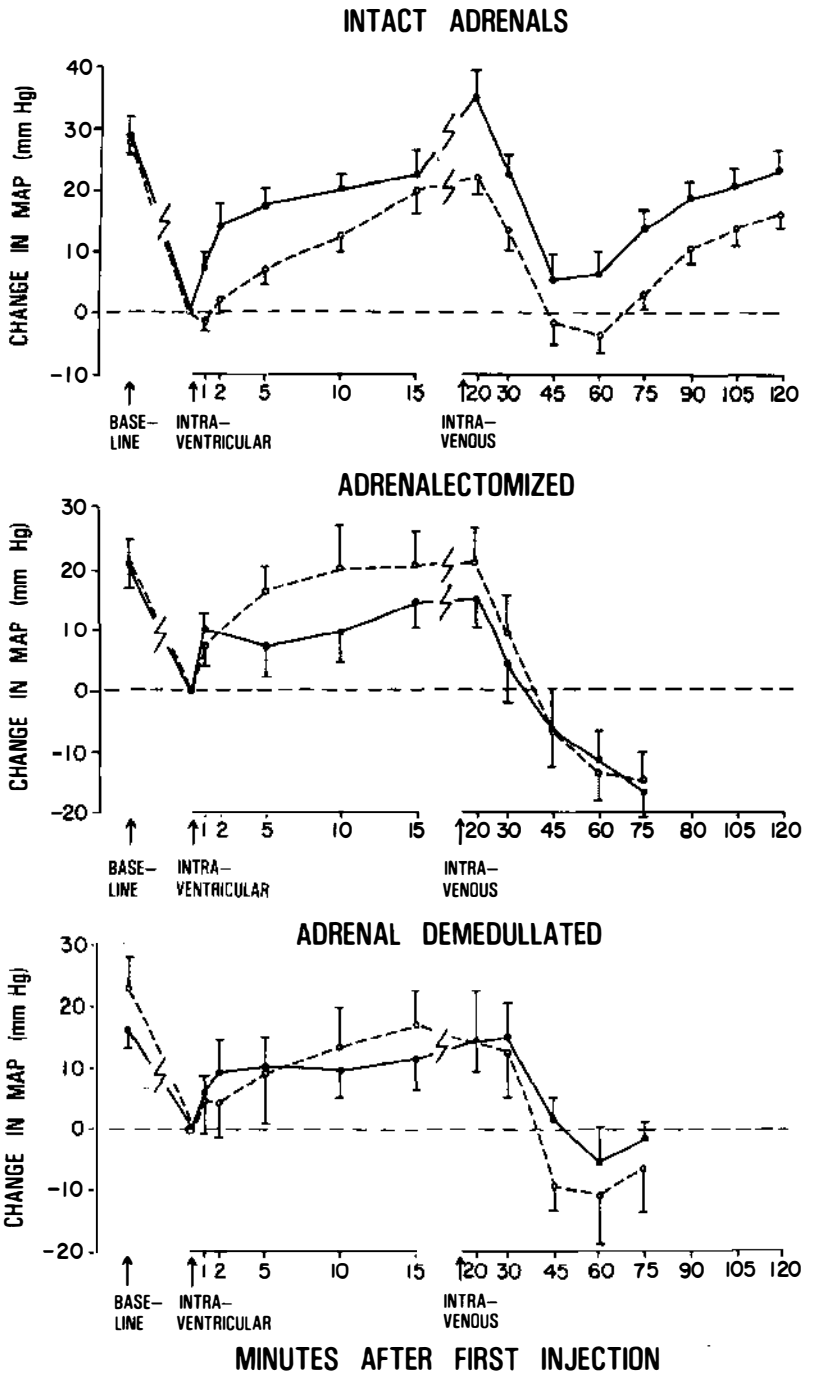
of naloxone's effects in reversing endotoxic shock hypotension (89), it is assumed that opiate antagonists exert therapeutic actions by displacing endogenous opiates from opiate receptors. Are these receptors located centrally or peripherally? Janssen & Lutherer (96) have reported that ventriculo-cisternal administration of naloxone protected against severe hypotension in dogs subjected to endotoxic shock. Their findings, indicating that naloxone improved circulatory function at sites within the brain, are in close agreement with studies from our laboratory, which have demonstrated a central nervous system action of naloxone in various shock models (18, 97, 98).

Rios & Jacob (99) used another approach to evaluate the site of naloxone's actions. These investigators demonstrated that the iodomethylate of naloxone, which does not readily cross the blood-brain barrier, failed to reverse endotoxic hypotension in rats upon peripheral injection, whereas central injection of this molecule produced the anticipated therapeutic effects. This collective evidence indicates an important central nervous system component in the therapeutic effects of naloxone in endotoxic shock. However, peripheral actions of naloxone, independent of its central effects, cannot be ruled out from these data.

Given that naloxone exerts central actions in reversing endotoxic hypotension, what peripheral effector organs are involved in mediating these autonomic responses? We conducted a series of studies to evaluate the potential role of the adrenal gland and sympatho-medullary responses in hemodynamic changes following central naloxone administration (97). It was found that total adrenalectomy and selective adrenal demedullation (wherein adrenal cortical function remained intact) both dramatically enhanced endotoxic shock susceptibility by greater than 15-fold. Moreover, both procedures completely blocked the pressor response to centrally or intravenously administered naloxone in conscious rats subjected to endotoxic hypotension (Figure 4).

These studies provide important clues as to the origin of the endogenous opiates that contribute to shock hypotension, as well as the mechanisms by which the improved circulatory function produced by opiate antagonists is mediated. The results indicate that adrenal enkephalins contribute little to the cardiovascular pathophysiology of endotoxic shock. Had adrenal enkephalins been involved, their surgical removal should have protected against the adverse cardiovascular effects of endotoxemia.

We also found that the beneficial cardiovascular effects of naloxone appear to involve central nervous system actions that are peripherally mediated by sympatho-medullary discharge. Finally, since it was shown that these adrenal demedullated rats had normal glucocorticoid levels, the importance of endogenous corticosteroids in shock susceptibility appears to be



secondary to the importance of adrenal-medullary actions. We concluded that endogenous opioid systems act at sites within the central nervous system to inhibit sympatho-medullary discharge. Naloxone antagonizes these central actions of endogenous opiates, thereby improving cardiovascular function through a sympatho-medullary action (97).

Consistent with these findings, Dashwood & Feldberg (100) have shown that the pressor response to naloxone following extensive surgical stress in cats was blocked by adrenalectomy and attenuated by cutting splanchnic nerves. Furthermore, Manugian et al (101) have found that intravenous or intracisternal naloxone injections produced an increase in preganglionic splanchnic nerve activity that was associated with an elevation in arterial pressure in anesthetized cats. Wright (102) has also obtained evidence for an adrenergic component of naloxone responses in endotoxemia. At variance with these observations, however, van Loon et al (49) have demonstrated that β-endorphin injections intracisternally produced increases in plasma epinephrine, norepinephrine, and dopamine in unanesthetized rats. Although hemodynamic variables were not measured by these investigators, such a response would predict an increase in arterial pressure following the apparent activation of sympathetic outflow by β-endorphin. Once again, however, it must be noted that pharmacological responses to opiate agonist injections may result in a confusing array of effects that have little bearing upon physiologically activated endogenous opiate systems and their concomitant cardiovascular function.

As opiate antagonists appear to improve the pathophysiology of endotoxic shock, we wished to determine whether opiate agonist administration would worsen shock hypotension. β-endorphin or morphine were injected intravenously following endotoxin in conscious rats (65). Both of these opioid agonists resulted in a further depression of arterial pressure when compared to the hypotensive effects of endotoxin alone. Moreover, the additive hypotensive effects of opioid agonists plus endotoxin were reversed by subsequent naloxone treatment. Recent work by Gahhos et al (94) has shown that morphine injections exacerbate the shock hypotension produced by live *E. coli* injections in pigs.

Figure 4 Following endotoxic shock hypotension, the effects of subsequent naloxone treatment upon mean arterial pressures (MAP) are compared among rats with intact adrenals (top), adrenalectomized rats (middle), and adrenal demedullated rats (bottom). Solid lines depict naloxone-treated animals, dashed lines represent saline control rats; n=10 or more per group, and vertical bars are ± SEM. Less than 1/15 of the dose of endotoxin used in intact (control) rats produced comparable hemodynamic effects in adrenalectomized and adrenal demedullated rats. Note that intraventricular naloxone (30 μg) followed by 3 mg/kg intravenous naloxone restored MAP only in rats with intact adrenals. Adrenalectomy and adrenal demedullation block naloxone's effects; see text and (97).

The finding that injection of opioid agonists enhances shock susceptibility provides no information as to which specific subpopulation of opioid receptors may be involved in mediating the endogenous opiate component of endotoxemia. Studies reviewed above (57) indicated that the injection of delta opioid agonists into the third ventricle of anesthetized rats produced a depression of mean arterial pressure and pulse pressure without altering heart rate in a manner qualitatively similar to hemodynamic responses observed in endotoxic shock models. Because of these similarities, we speculated that endogenous opioids acted upon delta receptors to produce their cardiovascular effects. Recently the availability of the delta antagonist peptide M154,129 has provided the opportunity to test this hypothesis (69). Indeed, we found that M154,129, at a dose of 30–60 mg/kg iv, selectively reversed endotoxic hypotension without altering the analgesic effects of simultaneously administered morphine (103). We have also observed that the mu_1 antagonist naloxazone was without effect in blocking or reversing endotoxic shock hypotension in the rat. More recently, we found that pretreatment with the selective, long-lasting mu antagonist β-FNA [see Table 1 and (68)] had no effect upon the pattern of change in arterial pressure induced by endotoxin in conscious rats (Figure 5; in preparation).

Thus, the predominant hemodynamic effects of endogenous opiates in endotoxic shock appear to be mediated by their action at delta opioid receptors. The future development of potent delta antagonists may provide a selective clinical tool by which to reverse shock without affecting morphine analgesia in humans.

Since adrenal enkephalins do not appear to be of primary importance in endotoxic shock hypotension, which of the endogenous opiate systems are involved? Adrenalectomy has long been known to elevate circulating levels of β-endorphin because of the lack of corticosteroid feedback at the level of the pituitary (104). It therefore appeared possible that pituitary endorphins were involved. Indeed, we have shown a five- to ten-fold increase in circulating β-endorphin levels during endotoxic shock in monkeys (Gurll, et al, in preparation). However, demonstrations of elevations in circulating endorphins along with naloxone reversibility of shock hypotension do not necessarily link these observations at a cause-and-effect level. In fact, although β-endorphin levels are dramatically increased in adrenalectomized animals, naloxone was shown to have no effect on endotoxic hypotension following adrenal extirpation in rats (97).

From the above studies with adrenalectomized animals, it may be concluded that neither adrenal enkephalins nor pituitary β-endorphin are primarily responsible for the naloxone-reversible component of endotoxic shock. The hypothesis that is most difficult to disprove supports a functional role for central enkephalins and/or β-endorphin pathways that act through

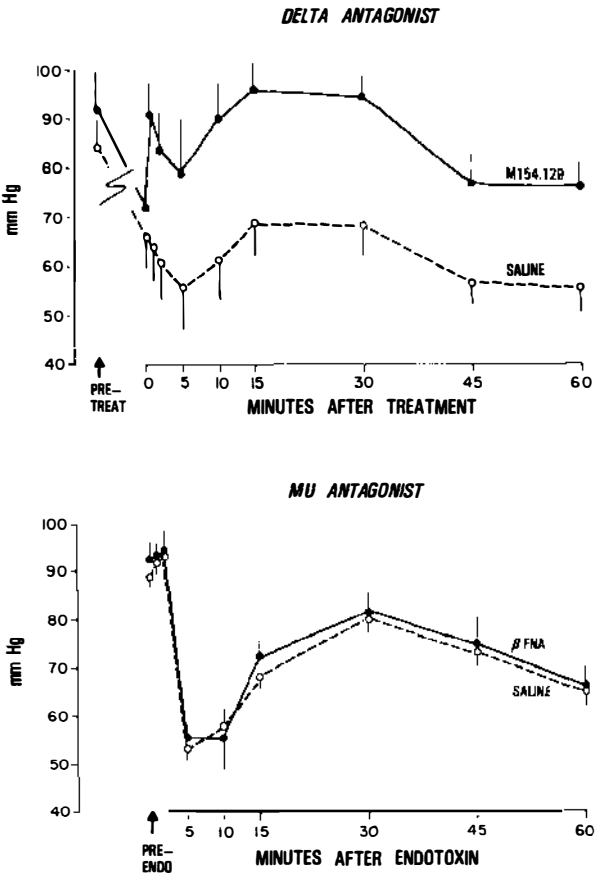


Figure 5 Comparison of the effects of a selective delta antagonist (M-154,129) with a selective mu antagonist (β-FNA) following the induction of endotoxic shock hypotension in conscious rats. The delta antagonist was administered at a dose of 60 mg/kg iv at time "0" on top graph; since β-FNA is a long-lasting mu antagonist, this substance was delivered intraventricularly at a dose of 10 μg 24 h before inducing shock. This dose of M-154,129 failed to alter morphine-induced (4 mg/kg) analgesia in rats, whereas this dose of β-FNA successfully abolished the analgesic effects of 8 mg/kg morphine. Dashed lines represent saline-treated rats; vertical bats are SEM. At least 8 rats/group are represented. These data indicate that endotoxic shock hypotension in the rat is mediated by actions of endogenous opiates acting at delta receptors; see text and (103).

sympathetic mechanisms to decrease sympatho-medullary outflow and thus contribute to the cardiovascular pathophysiology of endotoxic shock. This tentative conclusion requires further experimental verification through manipulations of central and peripheral β-endorphin and enkephalin systems.

Summary The pathophysiology of endotoxemia appears to involve an important endogenous opiate component that is reversed by the opiate antagonist naloxone. Among opiate antagonists studied to date, naloxone, naltrexone, and M154,129 have all been shown to improve circulatory function in endotoxic animals; naloxazone, a mu_1 antagonist of opioid receptors, failed to alter endotoxic hypotension. Evidence obtained with M154,129, an antagonist with a selective action at delta opioid receptors, indicates that shock hypotension may be mediated by endogenous opiates acting at this specific receptor subtype. The therapeutic efficacy of opiate antagonists has been experimentally confirmed in mice, rats, cats, dogs, sheep, pigs, horses, monkeys, and man. A series of anecdotal reports has appeared in the literature indicating that patients suffering from septic shock experience an improvement in hemodynamic variables following administration of naloxone (105–111). Naloxone has been shown to improve circulatory variables, particularly cardiac contractility, which was also associated with an increased tissue perfusion and survival. In addition to cardiovascular effects, naloxone also affected body temperature, improved white blood cell and platelet counts, attenuated hypoglycemia, prevented acidosis, and improved survival. These therapeutic effects of naloxone seem to be largely a consequence of actions at opiate receptors within the brain, and intact sympatho-medullary function appears essential for their expression. Acidosis and hypothermia significantly blunt the response to naloxone in experimental endotoxemia (vide infra). Finally, the simultaneous administration of opiate analgesics along with endotoxic hypotension results in a further exacerbation of the syndrome. Thus, opiate analgesics should be used with caution in clinical situations of septic shock.

HEMORRHAGIC SHOCK It was important to ascertain whether the endogenous opiate component of circulatory shock was unique to endotoxin interactions or more general in nature. To evaluate the hypothesis that endogenous opiates are fundamentally involved in the etiology of a variety of shock states, we studied the effects of naloxone injections in conscious rats subjected to hemorrhagic shock following the rapid removal of 50% of their estimated total blood volume (17).

The intravenous administration of 1 mg/kg naloxone was shown to improve significantly mean arterial pressure and pulse pressure in these hypovolemic rats. Moreover, survival in the naloxone-treated group was significantly improved when compared to saline-control animals (17). Subsequent studies with Vargish et al (111) provided further insights into these therapeutic effects of naloxone in dogs subjected to hemorrhagic shock. In those studies, naloxone (2 mg/kg followed by 2 mg/kg per hour) resulted in a significant increase in inotropic function, which was reflected in eleva-

tions of cardiac output and mean arterial pressure. As with the endotoxic studies, naloxone did not alter heart rate, total peripheral resistance, or portal venous pressure. When this severe model of hemorrhagic shock was used in dogs, all the saline-treated animals died, while all the naloxone-treated dogs survived (111).

Recently, Gurll and colleagues (112, 113) have shown that these therapeutic effects of naloxone are dose-related. Even without blood reinfusion, naloxone improved hemodynamic variables as well as survival in a dose-related way. Further studies in their laboratory (114) have shown that naltrexone, an opiate antagonist with a longer duration of action, also resulted in a significant improvement in hemodynamic variables as well as survival in this canine hemorrhagic shock model.

These hemodynamic effects of naloxone in dogs have been confirmed by Toth et al using less invasive measurement procedures (115). These investigators reported results similar to those described by Vargish et al (111); however, they obtained evidence that naloxone may increase venous return as well as improve stroke volume, cardiac output, and mean arterial pressure.

In studies with anephric cats as well as cats with intact kidneys, Feuerstein et al (116) have shown that 0.1 mg/kg per minute of naloxone promoted a sustained improvement in arterial pressure following acute hemorrhage. Curtis & Lefer (117) have also studied hemorrhagic shock in cats; they have reported that similar doses of naloxone had beneficial effects upon hemodynamic and metabolic variables. Furthermore, naloxone was demonstrated to reduce lysosomal enzyme release and to depress circulating concentrations of myocardial depressant factor (MDF), a toxic pancreatic peptide released in shock. However, MDF is not an endogenous opiate as these investigators failed to find an effect of naloxone in reversing the myocardial depression produced by MDF in vitro (117).

Schadt & York (118) have demonstrated therapeutic effects of naloxone in a conscious rabbit hemorrhagic shock model. These investigators observed a dose-related increase in mean arterial pressure, accompanied by a significant decrease in heart rate. In cynomolgus monkeys subjected to hemorrhagic shock, naloxone was shown to improve significantly circulatory function and survival (119). Moreover, in these collaborative studies with Gurll and colleagues (in preparation), plasma β-endorphin and β-lipotropin concentrations were demonstrated to be significantly elevated following the onset of hemorrhagic hypotension.

Mechanisms of naloxone's actions in hemorrhagic shock In studies designed to assess the sites of naloxone's action as well as the origin of endogenous opiates involved in hemorrhagic shock hypotension, experi-

ments were conducted with hypophysectomized and sham-operated rats (98). Following the induction of hemorrhagic shock, naloxone was initially injected intracerebroventricularly, followed by a later intravenous dose. As with endotoxic shock, low doses of naloxone (10.0 μg) injected into the ventricular spaces of the brain resulted in a significant improvement in mean arterial pressure in sham-operated animals with intact pituitary glands, indicating a central site of action for naloxone in this model.

If pituitary endorphin were the endogenous opiate responsible for the naloxone-reversible component of hemorrhagic shock hypotension, one would expect naloxone to have no effect in animals that lack pituitary endorphin owing to hypophysectomy. Indeed, this was the case; hypophysectomy blocked the beneficial response to naloxone following either intracerebroventricular or intravenous injection (98). However, this simplistic interpretation must be reevaluated in light of the atrophic effects of hypophysectomy on secondary endocrine systems. For instance, adrenal cortical and adrenal medullary function are severely compromised following removal of the pituitary gland (120). Patton et al (121) have found, as did we, that adrenalectomy abolished the effects of naloxone in canine hemorrhagic shock models. Thus, adrenal atrophy following hypophysectomy may be primarily responsible for the lack of naloxone's hemodynamic effects in hypophysectomized rats (98).

Investigations by Lang and colleagues (20) have demonstrated significant increases in circulating β-endorphin and enkephalin following hemorrhagic shock in the dog. However, increased circulating concentration of either of these endogenous opiates does not necessarily indicate their causal relationship to hemorrhagic hypotension. An important caveat must be remembered: the actual loss of blood following hemorrhage or its functional loss by sequestration during other forms of shock will result in a lower absolute volume in which to distribute any endogenously released substance or pharmacologically administered drug. If a gland secretes a hormone at a constant rate, and the functional blood volume decreases by half, as occurs in many hemorrhagic shock models, an apparent doubling of hormonal concentration will occur. Likewise, any drug administered intravenously on a basis of mg/kg total body weight will be dissolved in a lesser volume of circulating blood in an organism subjected to shock. Thus, the effective concentration of blood-borne hormones as well as injected drugs will be higher than in the normovolemic animal.

Future evidence will likely indicate the importance of intact sympathomedullary function in mediating the improved hemodynamic effects of naloxone in hemorrhagic shock, as was previously shown in endotoxic shock. Although Feuerstein and colleagues (122) failed to observe significant plasma catecholamine elevations accompanying pressor responses to

naloxone injections in hemorrhagic shock, Schadt & York (118, 123) have demonstrated that the pressor effects of naloxone in their conscious rabbit model of hemorrhagic shock were mediated by α-adrenergic systems. Furthermore, these investigators reported an involvement of both sympathetic and parasympathetic systems following naloxone treatment, as β-adrenergic antagonists combined with the cholinergic antagonist atropine also prevented naloxone's therapeutic effects in this model.

An important criterion in showing involvement of endogenous opiate systems in physiologic states is the demonstration that different types of opiate antagonists exert similar effects (124). To this end, Gurll et al (114) have shown that naltrexone, like naloxone, improves survival as well as cardiovascular function in the canine hemorrhagic shock model. Chance et al (125) evaluated the effects of meptazinol, a compound with analgesic activity as well as opiate antagonist properties. These investigators found that meptazinol and naloxone both result in a rapid increase in mean arterial pressure following hemorrhagic shock in rats. By contrast, as observed in septic shock models (65, 94), morphine produces an immediate hypotension following the injection of analgesic dosages in hypovolemic animals (125). These results suggested that meptazinol could have combined analgesic properties and improve cardiovascular function in situations of hemorrhagic shock (125).

More recently, Curtis & Lefer have observed beneficial actions of a novel opiate antagonist Win 44,441 in hemorrhagic shock (126). This substance is likewise reported to have analgesic properties as well as opiate antagonist effects. Unlike naloxone, Win 44,441 failed to stabilize lysosomal membranes or to retard proteolysis in vitro. However, consistent with earlier findings (18, 89), only the active (–) isomer of Win 44,441 showed therapeutic effects in this model (126). Collectively, these results indicated that different opiate antagonists exert therapeutic effects that may be mediated at opioid receptors.

Summary Comparison of data between hemorrhagic and endotoxic shock models reveals a number of similarities regarding the putative involvement of endogenous opiate systems. Naloxone appears to act at sites within the central nervous system to reverse both forms of circulatory shock. This specific improvement in hemodynamic variables seems to be mediated predominantly by improved cardiac contractility as well as secondary improvement of metabolic variables and survival. As with endotoxic shock, hypothermia and acidosis significantly attenuate the pressor response to naloxone in monkeys subjected to hemorrhagic shock (127) (vide infra). At least four different opiate antagonists have been shown to have beneficial effects, and autonomic pathways are involved in mediating these responses.

Adrenalectomy blocks the therapeutic effects of naloxone in this model; however, hydrocortisone replacement restored naloxone's effects in hemorrhaged dogs (121). Since hydrocortisone delays catecholamine metabolism by inhibiting catechol-o-methyl transferase activity, this glucocorticoid could potentiate the vascular actions of sympathetically-released norepinephrine in hemorrhagic shock. However, the precise mechanisms and origins of endogenous opiates involved in the etiology of hemorrhagic shock hypotension remain to be determined. Although both plasma endorphins and adrenally derived enkephalins (20) are elevated in hemorrhagic shock, cause and effect cannot be directly related to these observations for the reasons mentioned above.

SPINAL "NEUROGENIC" SHOCK Acute transection of the cervical spinal cord has long been known to produce a condition known as "spinal shock." To a great extent, previous research in this area has emphasized changes in somatic reflexes without stressing the profound alterations in autonomic function produced by a rapid severing of the cervical spinal cord. We investigated the autonomic responses following acute cord transection and the effects of subsequent naloxone injection in an attempt to evaluate the possible generalization of endorphin involvement to a model of "neurogenic" shock (18, 128).

Using an anesthetized rat preparation, rapid transection of the cervical spinal cord at C6–67 resulted in a transient pressor response followed by a protracted secondary hypotension, with mean arterial pressure remaining 20–30 mm Hg below baseline. Injection of 10 mg/kg naloxone intravenously resulted in a rapid restoration of arterial pressure to pretransection levels.

Evidence supporting a central site of action for naloxone in this model was obtained by demonstrating that injection of as little as 48 μg of the (–) naloxone isomer (a dose that was ineffective upon intravenous injection) into the ventricular spaces of the rat brain following spinal shock hypotension produced a restoration of arterial pressure equivalent to that observed after injection of much larger doses of naloxone (10 mg/kg) intravenously. An equivalent amount of (+) naloxone was ineffective, confirming that naloxone's effects were stereospecific. Taken together, these results indicate that the hemodynamic responses to naloxone in spinal shock are mediated by opiate receptors within the central nervous system (18).

In this model, spinal transection also caused a decrease in respiratory rates and body temperature. Naloxone injections returned respiratory rates to pretransection levels and attenuated the hypothermia (18). This evidence indicates that, in addition to having cardiovascular responses, endogenous opioids are more generally involved in the centrally-mediated autonomic

dysfunction that follows acute cervical transection. Moreover, naloxone appears to improve survival following spinal cord transection in the rat (G. Nilaver, personal communication).

Parallel studies were conducted with anesthetized cats; as before, the effects of naloxone were observed when injected 45 min after acute cervical transection (128). At a dose of 2 mg/kg followed by infusion at 2 mg/kg/hh, naloxone resulted in a sustained improvement in mean arterial pressure in these animals as well.

Mechanisms and sites of naloxone's actions in spinal shock When the cervical spinal cord is severed, supraspinal (brain) control of sympathetic outflow is interrupted and only parasympathetic circuits are left intact to mediate the centrally-induced improvement in cardiovascular function following naloxone injection. The demonstration that naloxone was effective upon intracerebroventricular administration in this model (18) provided strong evidence that central parasympathetic systems are important in this form of circulatory shock.

Studies were conducted to evaluate the specific role of parasympathetic-vagal pathways in mediating the cardiovascular responses to naloxone in spinal shock. In rats and cats subjected to spinal shock, bilateral cervical vagotomy by itself improved arterial pressure following cord transection, which indicated that endogenous opiates may act centrally to enhance parasympathetic tone and thereby depress circulatory function in spinal shock (128). Subsequent injection of naloxone following vagotomy in spinal shock rats failed to result in a further improvement in hemodynamics, thus confirming that central opioid actions are mediated by parasympathetic pathways. Injection of muscarinic-cholinergic antagonists also blocked the hemodynamic improvement produced by naloxone in this model, a finding consistent with a cholinergic mediation of the parasympathetic effects of this opiate antagonist (128).

As with other shock models we have studied, naloxone failed to alter heart rate when injected following the onset of spinal shock hypotension. Evaluation of changes in left ventricular pressure over time (dp/dt) revealed that the decrease in contractility following cervical cord transection was specifically improved by naloxone (128). These data indicate that naloxone was producing an increase in left ventricular contractility. Although "classical" conceptualization of central cardiovascular control emphasized the effects of parasympathetic-vagal outflow upon heart rate, DeGeest et al (129) have provided convincing evidence that decreased contractility can occur without bradycardia when certain parasympathetic pathways are activated. Such a system would be compatible with our observations that naloxone administration following acute cervical spinal transection affected

parasympathetic mechanisms to improve contractility without altering heart rate.

The sources of endogenous opiates that contribute to spinal shock are poorly known. Central enkephalin or β-endorphin actions may be implicated by evidence that naloxone reverses the hypotension of spinal shock following intracerebroventricular injection (18). However, sympatho-medullary outflow may be an important contributor; the release of adrenal enkephalins by the currents of injury following cord transection could result in their actions upon pulmonary "J" receptors, thus affecting brainstem parasympathetic outflow (Figure 2). Therefore, it may be possible that naloxone acts upon peripheral afferent and/or central parasympathetic pathways to reverse the cardiodepressant effects of endogenous opiates at either site.

Summary The hypotension that accompanies acute transection of the cervical spinal cord appears to involve in part an action of endogenous opiates at receptors located at central parasympathetic centers; their activation results in an increase in vagal tone and decrease in cardiac contractility. Although enkephalin release from the adrenal medulla may play a part in the initial cardiovascular responses following acute spinal cord transection, sympathetic outflow is not directly involved in the therapeutic response to naloxone since the preganglionic effector pathways are severed by cervical cord transection. By contrast, endotoxic (and perhaps hemorrhagic) shock appears primarily to involve an endogenous opioid action upon central sympathetic nuclei. Thus, endogenous opiates appear to affect different autonomic pathways, which vary according to their differential activation in various forms of circulatory shock.

Endogenous Opiates and Spinal Injury

Cervical spinal cord injury, like complete transection, results in hemodynamic changes, including a loss of autoregulatory control of spinal cord perfusion and hypotension. The net result of these combined effects may be an exacerbation of spinal cord ischemia that follows traumatic spinal injury. We theorized that naloxone could improve spinal perfusion and ultimate neurologic recovery by opposing possible adverse effects of endogenous opiates following their activation by blunt injury to the cervical spinal cord (130). In anesthetized cats, spinal cord trauma was produced by the rapid application of a 500 g-cm force on exposed dura of the cervical spinal cord at C7. Naloxone injections 45 min following injury resulted in significant increase in arterial pressure as well as improved spinal cord blood flow to both gray and white matter (130, 131). Moreover, following spinal injury, plasma and cerebrospinal fluid levels of endogenous opiate materials were greatly increased (131).

The pressor response to naloxone injections following spinal cord injury may involve an activation of endogenous catecholamine systems following naloxone injection. In cats subjected to acute cervical cord transection, the pressor response to naloxone was accompanied by a selective increase in circulating concentrations of plasma dopamine; plasma epinephrine and norepinephrine concentrations were unaltered (132). To address whether the pressor response of naloxone was partially a consequence of the well-known cardiotonic effects of dopamine, the peripherally acting dopamine antagonist domperidone was administered following spinal cord injury but prior to naloxone treatment. Indeed, domperidone significantly attenuated the increases in cardiac contractility and arterial pressure produced by naloxone, thus indicating that endogenously released dopamine may contribute to the cardiovascular actions of naloxone in this model (132).

More importantly, naloxone administration, even when delayed as long as four hours following injury, significantly improved neurological recovery following severe spinal trauma (130, 131, 133). At six weeks post-injury, naloxone-treated cats showed only minor neurologic impairment, whereas the median saline-injected cats showed marked spastic quadriparesis. These results indicate an indirect, yet significant pathophysiologic role for the endogenous opiate system in the pathophysiologic consequences of spinal cord injury. Not only were endogenous opiate levels elevated at the time of maximum post-injury hypotension, but naloxone also improved spinal cord perfusion as well as ultimate neurologic outcome.

Recent work by Young et al (134) has confirmed our earlier results. Using a thoracic spinal injury model in cats, these investigators demonstrated that naloxone improved blood flow in the lateral column white matter and preserved somatosensory-evoked potentials when evaluated 24 h following injury and treatment (134). As we had observed, neurologic recovery was significantly better in the naloxone-treated animals. Collectively, these results confirm a possible role for endogenous opiates in the hemodynamic and neurologic sequelae following spinal trauma and indicate the potential clinical utility of opiate antagonists in the acute management of spinal cord injuries.

Endogenous Opiates in Cerebral Ischemia

As endogenous opiates appeared to be involved in the neuronal ischemia following spinal cord injury, we hypothesized that these substances could also contribute to the CNS ischemia following stroke or myocardial infarction. The gerbil has historically served as a model of cerebral ischemia as this animal has an incomplete circle of Willis. Since posterior communicating arteries are absent, and the anterior communicating artery may also fail to function in about one third of the animals, one can perform either

temporary or permanent carotid occlusion and induce reliable cerebral ischemia accompanied by neurologic deficits and ultimate death. We evaluated the cardiovascular and neurologic effects of naloxone in this gerbil model of stroke (135–137).

Using aneurism clips, the carotid arteries of anesthetized gerbils were bilaterally occluded for 30 min. Following the baroreflex-hypertension accompanying the blockade of carotid blood flow, release of carotid clips resulted in a naloxone-reversible hypotension. The effects of continuous naloxone treatment were compared to saline-treated controls with regard to functional neurological recovery six hours after clip release and subsequent drug initiation. Evaluations included assessment of time to awaken, respiratory rate, ptosis, circling behavior, locomotor and hot plate testing, righting reflexes, opisthotonus, seizures, and survival. An observer who was unaware of drug treatment groups found no therapeutic effects of naloxone on any of the above measures (135). Although naloxone improved blood pressure following clip release, an absence of subsequent neurologic improvements suggested that these two effects were dissociated (135).

Using a permanent unilateral carotid ligation, as opposed to the temporary bilateral carotid occlusion we evaluated, Hosobuchi et al (138) reported that naloxone improved neurologic recovery and survival in gerbils. We were unable to replicate these effects with naloxone (135–137). Although morphine injections did exacerbate the "neurologic" signs following carotid occlusion in both studies (135, 138), this result does not necessarily indicate a physiologic role of endogenously activated opiate systems in stroke.

The discrepancies between the responses to naloxone in these studies casts doubt upon endogenous opiate involvement in experimental stroke models that use the gerbil. However, recent work by Faden et al (139) has shown that naloxone improves somatosensory-evoked potentials in dogs following cerebral ischemia induced by carotid injections of air emboli. In clinical studies, Baskin & Hosobuchi (140) reported a transient naloxone reversal of hemiplegia secondary to cerebral ischemia in two patients who had no confirmed focal cerebral infarction. Since cerebral ischemia can result from many causes of both central and peripheral origin, and since species may show considerable variation, the issue of the involvement of endogenous opiate systems in cerebral ischemia and experimental stroke remains to be resolved.

Endogenous Opiates and Other Forms of Induced Hypotension

A variety of other investigations involving different models of induced hypotension have demonstrated that naloxone can improve arterial pressure. Paciorek & Todd (141) compared the cardiovascular effects of mep-

tazinol and naloxone after the induction of anaphylactic shock in anesthetized rats. Specifically, meptazinol was shown to be more effective than naloxone in improving mean arterial pressure following systemic anaphylactic shock produced by antigen injection in *B. pertussis*-sensitized rats. Recently, Amir has also demonstrated that opiate antagonists improve survival in anaphylactic shock (141a). Vargish et al (in preparation) have demonstrated that naloxone reverses the hypotension that follows burn shock in guinea pigs. Activation of an endogenous opiate-mediated hypotension by surgical stress was inferred by Dashwood & Feldberg (100). Those investigators observed that the more extensive the surgical procedures, the greater the pressor response following naloxone injection.

In work by Lind et al (142), the hypotension that follows occlusion of the superior mesenteric artery in anesthetized dogs was shown to be rapidly reversed by subsequent naloxone treatment. Using cats, Curtis & Lefer (143) have also demonstrated that naloxone improved arterial pressure and survival in splanchnic artery occlusion shock and prevented the accumulation of myocardial depressant factor that usually results from this procedure. More recently, Eddy and colleagues have shown that naloxone increased arterial pressure and plasma dopamine concentrations in canine splanchnic arterial occlusion shock (144), a finding that parallels our evidence indicating a possible dopamine involvement in cardiovascular responses to naloxone following spinal injury (132).

Huidobro-Toro & Mussachio (145) have reported that insulin-induced hypotension is reversed by naloxone in rats pretreated with reserpine. From this, they concluded that hypoglycemia caused a release of opiate-like material that mediated a hypotensive response. Recently, Goldstein et al (146) reported that naloxone attenuated the hypotension induced by Hageman factor, but failed to alter hypotension resulting from kallikrein, bradykinin, or nitroglycerin injections. Since Hageman factor has enzymatic properties, they surmised that this substance generated vasoactive-opioid peptides from circulating precursors.

Rubin and associates (147) have demonstrated that the fall in blood pressure during non-rapid eye movement sleep in humans is prevented by naloxone treatment. Although the hypotension that follows administration of certain anesthetics (e.g. pentobarbital) is not reversed by naloxone, Arndt & Freye (148) have demonstrated that hypotensive effects of halothane were stereospecifically reversed by naloxone perfusion of brainstem parasympathetic centers. More recently Artru et al (149) have confirmed these investigations with similar findings.

Alpha adrenergic antagonists, such as clonidine and α-methyldopa, have hypotensive actions that prompt their use in treating chronic hypertension. Experiments conducted by Farsang & Kunos (150) in rats, as well as clinical trials by Resnick et al (151), have demonstrated that clonidine's hypotensive

actions are reversed by naloxone. In addition, Farsang et al (152) have demonstrated that the α-methyldopa-induced hypotension is also naloxone reversible. Since research by Pettibone & Mueller (153) has shown that clonidine acts at the pituitary gland to increase the release of β-endorphin into the bloodstream, it is possible that naloxone is acting to reverse the depressor effects following a clonidine-induced release of β-endorphin (154).

Recently, Einhorn and colleagues (154a) reported that fasting in spontaneously hypertensive rats (SHR) produced a hypotensive state that was reversed by subsequent naltrexone administration; naltrexone was without effect in lowering the elevated pressures in non-fasted control rats. These interesting findings related appetitive and cardiovascular responses through common endogenous opiate systems.

Factors that Modify the Therapeutic Response to Naloxone in Hypotension

When the effects of early versus late naloxone administration in rats subjected to endotoxic shock were compared, naloxone produced a greater improvement in arterial pressure during the earlier stages of cardiovascular decompensation (155). This finding may be related to depletion of endogenous opiates as shock progresses, which diminishes their role in depressing circulatory function. Alternatively, a decrease in the efficacy of naloxone may be a consequence of an alteration in the physiologic milieu.

Blood pH is a critical factor in endotoxemia. Indeed, Raymond and colleagues (92) have shown that naloxone prevents the progressive acidosis that occurs in endotoxemic dogs. Recently, Rees et al (156) have found that naloxone reversed gastric epithelial hypoxia and prevented the development of systemic acidosis in a live organism model of *E. coli* sepsis in dogs. In these studies, naloxone treatment was initiated soon after the onset of endotoxic shock.

Does blood pH alter the effects of opiate agonists or antagonists? Kaufman has reported that acidosis severely attenuates the effects of both agonist and antagonist opiate substances (157). It would be expected then that naloxone's effects would be diminished in an acidotic subject. Indeed, Gurll et al (127) have shown in monkeys subjected to hemorrhagic shock that the greater the acidosis, the less the pressor response to naloxone. Therefore, it becomes critical that normal blood pH be restored if naloxone is to produce maximal therapeutic effects in the later stages of shock, which are characterized in part by systemic acidosis. Acidosis may limit blood-brain barrier penetration of peripherally administered naloxone; however, experimental confirmation of this hypothesis is lacking.

Ambient temperature has been shown to alter the cardiovascular response to opiate substances. Specifically, Janssen et al (158) have demon-

strated that the pressor response to naloxone in endotoxic hypotension is blunted by a cold ambient temperature. The specific reasons why changes in ambient temperature affect naloxone's responses are unknown, although it has long been understood that endotoxemic patients who become hypothermic have a generally poor prognosis for recovery.

We have speculated that one of the beneficial actions of glucocorticoids in shock could be their suppression of β-endorphin release via feedback mechanisms (16, 89). It was suggested that the resulting absence of β-endorphin would eliminate the endogenous opiate component of shock, thus leaving naloxone without any opiate effect to antagonize. Experimental evidence for this antagonism of naloxone's efficacy by high doses of steroids was obtained by Lutz et al (159). They observed that high doses of dexamethasone or methylprednisolone blocked the beneficial hemodynamic effects of naloxone in canine hemorrhagic shock. In agreement with this result, Peters et al (106) have reported that prior steroid treatment abolished the pressor response to naloxone in septic patients.

Recently, however, Weissglas et al have demonstrated additive effects of steroids and naloxone in pigs subjected to septic shock (160); Patton and colleagues (121) have shown that relatively low doses of hydrocortisone restore the therapeutic effects of naloxone in adrenalectomized dogs subjected to hemorrhagic shock hypotension. The dosage and potency of glucocorticoids used in these studies varied considerably, and discrepancies in results may be related to a higher dose suppressant effect and a lower dose potentiation of naloxone's therapeutic actions. Moreover, the initial speculation as to a primary role for pituitary endorphins in shock pathophysiology (16, 42, 98) must be reevaluated in light of the evidence that adrenalectomized rats failed to respond to naloxone following endotoxic shock despite greatly elevated circulating levels of β-endorphin-like immunoreactivity (97).

One important factor that appears to alter opioid agonist effects on the cardiovascular system is the presence or absence of simultaneous analgesia (vide supra). However, without exception, anesthetics have been shown to have little qualitative effect upon the beneficial hemodynamic responses to naloxone in various shock models across numerous animal species.

Endogenous Opiates and the Physiology of Baroreceptor Reflexes

As summarized above, one of the most widely accepted cardiovascular actions of injected opiate substances is their depressant effect upon baroreceptor reflex systems. However, the issue as to whether endogenous opiate substances are involved in the physiologic regulation of baroreceptor responses cannot be resolved by opiate agonist injections. Instead, we sought to activate endogenous baroreceptor reflexes through the use of transauricu-

lar electroshock in rats; the effects of opiate antagonism on subsequent cardiovascular responses were determined (161).

Electroconvulsive shock (ECS) resulted in an immediate 200% increase in arterial pressure, followed by a return to baseline pressure within 20 sec. The bradycardia that followed this surge in arterial pressure lasted 35 sec post-ECS. Naloxone pretreatment, at doses between 1 and 10 mg/kg, produced an exaggerated hypotensive swing accompanied by an enhancement of reflex bradycardia in these animals. From these results, we concluded that baroreflex activation by nonpharmacological, noninvasive means results in the functional recruitment of endogenous opiate systems. The finding that naloxone exaggerated the reflex cardiovascular responses indicated that endogenous opiate systems provided a physiologic "buffering" of the usual reflex swings in heart rate and arterial pressure which was blocked by naloxone pretreatment (161).

Other investigators have used pharmacologic means to induce rapid arterial pressure increases in order to study subsequent baroreflex responses and the effects of naloxone treatment. In studies by Petty & Reid (74), both pressor (phenylephrine) and depressor (sodium nitroprusside) substances were used to activate baroreceptor responses, and reflex heart rate effects were reduced following injection of an enkephalin analog and enhanced by naloxone treatment. Similar results have also been obtained by Freye & Arndt (75), Montastruc et al (76), and Sander et al (64). Thus, there is strong physiological and pharmacological evidence that endogenously activated opiate systems blunt the baroreceptor reflex responses in a number of species. Since orthostatic hypotension is an important neurologic problem in certain autonomic diseases and following nonopiate drug treatments, an endorphin involvement in the etiology of such baroreceptor-involved syndromes may have clinical relevance.

Endogenous Opiates and Chronic Hypertension

The data reviewed in Section 1 indicate that opioid peptides may have hypertensive properties that are mediated via opiate systems as well as nonopiate systems. The work of Farsang & Kunos (150, 152, 154) and others has demonstrated that the hypotensive actions of clonidine are reversed by naloxone. Since clonidine functions as an anti-hypertensive drug, and since opioid substances can produce hypertension following their pharmacological administration, does this finding indicate a possible role of endogenous opiate systems in essential hypertension?

Several lines of evidence suggest that this is not an important role for endogenous opiates. For example, in unanesthetized animals, the increase in arterial pressure produced by enkephalin has been reported to be resistant to naloxone reversal (47). More importantly, naloxone has been shown to

be without effect upon elevated blood pressure in spontaneously hypertensive rats, in rats rendered hypertensive by unilateral renal-arterial occlusion, or in hypertension produced by deoxycorticosterone acetate administration (50, 162, 163).

The absence of a naloxone-reversible response following physiologically- or pharmacologically-induced hypertension may reflect the relative inability of naloxone to act upon the specific opiate receptor subtype (e.g. delta) that could be more primarily involved in the hypertensive effects of endogenous opiates. As noted above, this possibility is doubtful as adequately high doses of naloxone were used (10.0 mg/kg) to overcome its relatively lower affinity for delta receptors (163).

Schaz and colleagues (63) have shown that the hypertensive effects of Leu-enkephalin were magnified in spontaneously hypertensive rats. This phenomenon may relate to the observation by Martucci & Hahn (164) of a doubling of the number of opiate binding sites coincidental with the onset of elevated arterial pressure in developing hypertensive rats. Yukimura et al (52) have recently shown that the opiate receptor antagonist diprenorphine produces a greater hypotension in spontaneously hypertensive rats than in normotensive control animals. Importantly, however, diprenorphine has agonist properties; it is not a pure opiate antagonist (A. Cowan, personal communication). Thus, the magnification of the depressor responses to diprenorphine in spontaneously hypertensive rats may only reflect an exaggerated hypotensive response to the agonist actions of this drug.

From the data summarized above, the relevance of endogenous opiate systems to the maintenance of chronically elevated arterial pressures seems in doubt. This important issue requires further experimentation when more specific and selective opiate receptor antagonists become available. However, it appears possible to extrapolate from the experimental results of Dworkin et al (165), that endogenous opiate systems may be involved in the generation (as opposed to maintenance) of chronically elevated pressures. Those investigators theorized that some hypertension may begin as an instrumentally learned blood pressure response for which the reward is a baroreceptor-mediated reduction in the aversiveness of ambient noxious stimuli. Experimental support for this hypothesis can be obtained from the work of Zamir and colleagues (162, 163), who have demonstrated that, accompanying the increase in arterial pressure following experimentally induced hypertension, a naloxone-reversible increase in analgesic latencies also occurs.

These collective data with SHR animals generally reveals that the hypertension associated with this trait is not altered by opiate antagonists; however, hypotension induced by fasting these animals is opiate-antagonist reversible (154a). Thus, appetitive, antinociceptive, and cardiovascular re-

sponses in SHR rats all may involve an endogenous opiate component in "reward" responses associated with these systems.

THYROTROPIN RELEASING HORMONE: A PHYSIOLOGICAL ANTAGONIST OF ENDOGENOUS OPIATE SYSTEMS

The potential therapeutic utility of receptor level opiate antagonists in reversing shock and preventing neurologic deficits has been summarized in the preceding sections. One of the concerns about the use of nonspecific opiate antagonists in treating these conditions is that their effects in blocking endogenously or exogenously produced opiate analgesia could impair their clinical utility in the treatment of traumatic injury. For example, although shock-like states could be reversed by these substances, intensification of pain may occur, and opiate analgesics would have no effect in alleviating that response. One approach to this issue has been to evaluate the possible involvement of specific opiate receptor subtypes in reversing shock without altering opiate analgesia. Indeed, we have shown that M154,129, a selective delta antagonist, has these properties (103) (vide supra).

An additional way to reverse shock and improve outcome in these experimental models has involved the use of thyrotropin releasing hormone (TRH), a tripeptide that does not bind to opiate receptors, yet antagonizes many of the biological effects of endogenous opiates without altering analgesic latencies (167). In earlier work, we and others demonstrated that TRH functionally antagonized opiate-induced catalepsy, hypothermia, and other behavioral responses (167, 168). Given these results, it appeared possible that TRH could have therapeutic value in experimental models of endotoxic and hemorrhagic shock as well as acute spinal cord injury.

In conscious animals, TRH (2 mg/kg iv) produced an increase in arterial pressure, heart rate, and respiratory rate (169). Thus, unlike naloxone, TRH has inherent cardiorespiratory effects of its own, which elevate these autonomic variables. When injected following experimental endotoxic or hemorrhagic shock, TRH, in a dose-related way, improved arterial pressure, pulse pressure, heart rate, respiration rate, and survival (Figure 6) (170). In fact, TRH was more efficacious than naloxone, and its therapeutic effects, like those of naloxone, appeared to be mediated in part through central effector sites that regulate sympatho-medullary outflow (97). Unlike those of naloxone, however, the hemodynamic effects of TRH may also involve direct peripheral actions (97). Doses of TRH that produced the improved cardiorespiratory responses were further shown to have no effect upon nociceptive latencies in rats or monkeys. In fact, when combined with

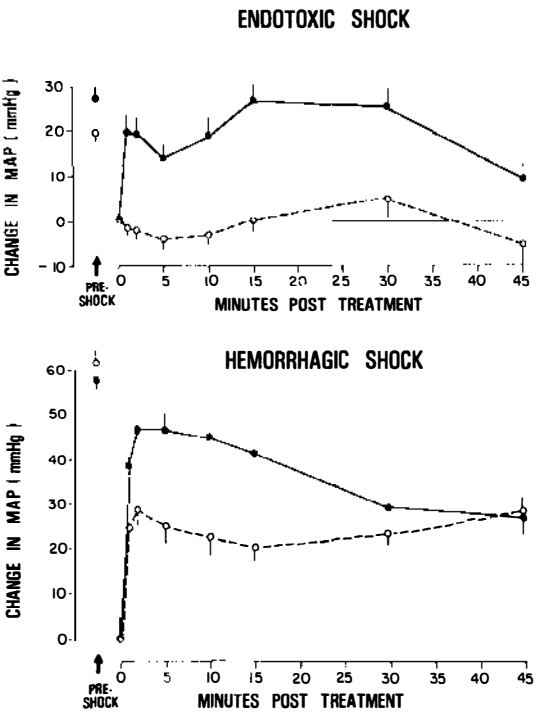


Figure 6 The effects of thyrotropin releasing hormone (TRH) are compared in rats subjected to endotoxic shock (top) and hemorrhagic shock (bottom). TRH, at an intravenous dose of 2 mg/kg, rapidly reversed the fall in arterial pressure produced by these two procedures. By contrast, saline injections had no effect in endotoxemic rats; however, this volume of saline partially improved MAP in hemorrhaged rats. This dose of TRH was shown to have no effect upon nociceptive latencies in rats or monkeys, nor did it antagonize morphine analgesia in these species. Again, vertical bars are SEM. At least 8 rats were used in each group (see 168).

analgesic doses of morphine, the antinociceptive properties were slightly enhanced by TRH (B. Cuthbert and J. W. Holaday, unpublished).

Given the successful results in the endotoxic and hemorrhagic shock models, we evaluated the efficacy of TRH in improving the neurologic outcome following experimental spinal injury in anesthetized cats (170). TRH was shown to be even more efficacious than naloxone in this model; survival following acute spinal injury was also significantly improved by this tripeptide (170).

In the gerbil model of cerebral ischemia, unlike the model of spinal injury, TRH was without any therapeutic effects (135). In fact, neurologic signs and survival were worsened in animals receiving TRH when compared to saline-injected gerbils. From this observation, we can conclude that mech-

anisms of ischemic responses in spinal injury and in stroke do not respond in a similar manner to TRH.

The effects of TRH in antagonizing cardiovascular and other responses to endogenous opiates appear to be mediated through opposing physiologic systems that utilize different receptors. Such an interaction is not unprecedented; the antagonist interactions between epinephrine and histamine in vivo are also mediated through opposing physiological systems. It is important to note, however, that TRH is not a selective antagonist for endogenous opiates. Nemeroff and colleagues (171) have reported that TRH effectively opposes the majority of responses evoked by neurotensin, as well.

The possible secondary involvement of pituitary thyroid responses in mediating TRH effects are of less importance. Several lines of evidence support this observation, including the lack of increased triiodothyronine levels following acute injections of large doses of TRH in experimental animals (172). Also, very small doses of TRH analogs injected intracerebroventricularly are therapeutically effective in shock models without altering peripheral endocrine status (J. W. Holaday, unpublished).

A comparison of the effects of TRH with opiate receptor-level antagonists such as naloxone in the various models of shock and spinal injury described above reveals that TRH has distinct therapeutic advantages over these substances. Not only is it more efficacious, but it does not appear to interfere with the analgesic system; it thus allows for concomitant opiate injections for pain relief. The effects of TRH appear to be mediated at central neural effector sites involving sympatho-medullary outflow, and via peripheral actions at unknown locations that also contribute to its pressor responses.

PHYSIOLOGICAL PERSPECTIVE AND CONCLUSIONS

The availability of the opiate antagonist naloxone has provided an opportunity to evaluate the possible involvement of endogenous opiate systems in a number of physiological responses (7). Although it is with reluctance that pharmacologists refer to a compound as "specific" or "pure," naloxone appears to be a good candidate for these titles. In addition to naloxone blockade, several other criteria have been offered by Sawynok and colleagues (124) in confirming an involvement of endogenous opiate systems in physiologic mechanisms. Almost all of these criteria have been met in studies involving the use of opiate antagonists in experimental shock and spinal injury. Others, such as demonstrating cross-tolerance between the physiological (or behavioral) state versus chronic opiate administration, have yet to be investigated. Indeed, this criterion may not be an absolute

prerequisite (173). However, evidence summarized above provides strong reasons to invoke an involvement of endogenous opiate systems in pathophysiological states such as shock and spinal injury as well as in "normal" physiological responses such as baroreceptor reflexes.

Which of the endogenous opiates may be involved in etiology of shock remains to be conclusively demonstrated. However, our evidence indicates that adrenal enkephalins or pituitary endorphins may be of lesser importance in these syndromes (97). The site of action of endogenous opiates also remains to be shown. However, evidence from our laboratories and others indicates a strong central nervous system component to naloxone responses that may involve both sympathetic and parasympathetic mechanisms. Specifically, naloxone works at CNS sites to improve hemodynamics following endotoxic, hemorrhagic, and spinal shock following the intraventricular injection of doses that are too low to have an effect when administered peripherally. In endotoxic shock, sympatho-medullary outflow appears important to naloxone's effects, whereas in spinal shock, parasympathetic-vagal outflow may be primarily involved.

Evidence was provided that the syndrome of shock is qualitatively similar to cardiovascular effects following third ventricular injection of a delta agonist in anesthetized animals. However, more specific evidence as to the possible involvement of delta opioid receptors in shock pathophysiology was obtained by the use of M154,129. This delta antagonist peptide effectively reversed endotoxic hypotension without altering morphine analgesia (103). Another approach to the problem of reversing shock without enhancing pain has been through the use of TRH, a tripeptide that opposes many of the effects of opiates, shock, and spinal injury at doses that fail to alter analgesic latencies.

The apparent common association of endogenous opiate systems with pain mechanisms as well as shock pathophysiology has provoked many philosophical questions. Historically, however, pain and shock have been closely linked. For example, prior to the 1840s, anesthetics were unavailable to alleviate the excruciating pain of surgical procedures. Surgeons were then judged by the speed of their operations since any delays in surgical procedures could result in fatal circulatory shock associated with the pain of the incision and blood loss. Observations obtained while treating wounded soldiers also emphasize the relationship between circulatory shock and pain. In the early 1900s, Cannon reported (174) that wounded soldiers often experienced circulatory shock that was more intense than superficially indicated by the severity of their wounds. Three decades later, Beecher (175) reported that soldiers were often without pain following severe injuries.

The discovery of the endogenous opiate receptors and their natural ligands has provided us with an opportunity to link pain and shock at a

functional level. It is now relatively well established that severe stress activates endogenous opioid systems to decrease the aversiveness of noxious stimuli as well as to contribute to the pathophysiology of shock. Teleologically, however, why would a system evolve to provide pain relief and then contribute to the demise of an organism through the induction of circulatory shock? Such an apparently maladaptive response is difficult to justify from a perspective of selective evolutionary advantages. Thomas (176), in his essay "On Natural Death," allows the possibility that the existence of endogenous opiates could provide for a pain-free death in the face of extreme injury. Although such a mechanism has superficial appeal, the pressures of natural selection would not favor such an evolutionary development.

We have speculated that the loss of blood pressure following severe pain may provide a mechanism by which blood loss through wounds would be minimized, thus allowing for coagulative process to seal the area and prevent further hemorrhage (177, 178). Alternatively, it is possible that this "maladaptive" response merely reflects an evolutionary imperfection that persists despite apparent negative selective pressures. Nonetheless, these arguments fail to provide a wholly satisfactory explanation. One way to rationalize the issue of "adaptive" endogenous opiate responses to pain relief and "maladaptive" responses in circulatory shock is to invoke the speculation that different endogenous opiate systems are involved in these two respects. Specifically, preliminary evidence indicates that shock is mediated by delta opioid receptors and analgesia is mu receptor-mediated. These two receptor subpopulations may therefore be independently activated (or inactivated) to allow for a selectivity of responses.

In a few short years, it has been demonstrated through anatomical, pharmacological, and physiological experimentation that endogenous opiate systems may profoundly affect circulatory function through central and peripheral actions that predominantly involve the autonomic nervous system. Research linking endogenous opiate systems to cardiovascular function has demonstrated their possible involvement in circulatory shock, spinal cord injury, baroreceptor reflex responses, hypertension, cerebral ischemia, and hypotension following the administration of certain anesthetic agents. These collective observations provide novel insights that indicate an opioid involvement in the physiological mechanisms that mediate the autonomic regulation of circulation.

Perhaps of greater importance, these experimental products of opiate research have great potential for extrapolation to the clinical environment. Opiate antagonists, including those with receptor level actions such as naloxone and compounds such as TRH, may be shown to have significant therapeutic value in treating shock due to bacterial infection, hemorrhage,

anaphylaxis, or even neurogenic causes. These substances also have potential utility in the therapy of spinal injury, stroke, orthostatic hypotension, and perhaps hypertension as well.

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