The Results of Exposure to Immobilization, Hemorrhagic Shock, and Cardiac Hypertrophy on β-Endorphin in Rat Cardiac Tissue (43730)

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Abstract. **In the present study, β-endorphin (BE), β-lipotropin (B-LPH) and the ratio of β-endorphin to β-lipotropin (BE:B-LPH) were determined in rat cardiac tissue in response to physical stress induced by immobilization and cardiovascular stress resulting from hemorrhagic shock and pressure overload-induced cardiac hypertrophy. As compared with controls, BE was increased and B-LPH was decreased in cardiac tissue from animals subjected to immobilization, and there was also a significant rise in the ratio of BE:B-LPH. Cardiac BE remained unchanged following hemorrhage, while B-LPH was diminished, resulting in an increase in the ratio of BE:B-LPH. Similarly, the concentration of BE was unchanged, the concentration of B-LPH was significantly diminished and the ratio of BE:B-LPH was significantly increased in hypertrophied hearts. Thus, immobilization-induced stress, hemorrhagic shock, and cardiac hypertrophy all increased the ratio of BE:B-LPH in the heart. However, it appears that immobilization stress induces an increase in cardiac BE, whereas cardiovascular stress results in a preservation of BE in the heart and a reduction in cardiac B-LPH. The data suggests that physical stress (induced by immobilization) and cardiovascular stress (i.e., hemorrhage, hypertrophy) have differential effects on the synthesis of BE and the post-translational processing of proopiomelanocortin in the heart. Furthermore, the alterations in cardiac tissue BE and possibly B-LPH may play a role in the response of the heart to physical and cardiovascular stress.** [P.S.E.B.M. 1994, Vol 206]

β-endorphin (BE) and β-lipotropin (B-LPH) have been localized in cardiac tissue of the rat (1). The synthesis of BE and B-LPH results from the enzymatic cleavage of the prohormone, proopiomelanocortin (POMC). Since BE comprises the terminal 31 amino acid portion of the B-LPH molecule, it can also be produced by the enzymatic cleavage of a molecule of B-LPH (2). BE and B-LPH synthesis in the heart is suggested by the presence of the messenger ribonucleic acid (mRNA) which codes for the BE portion of the POMC molecule (3). BE produced in cardiac tissue may be secreted locally, exerting a paracrine modulation of heart function. The participation of opiates in the regulation of cardiac function is suggested by the presence of opiate receptors in the heart (4) and by the observation that administration of BE to isolated-perfused hearts results in a modest decrease in the left ventricular systolic and diastolic pressure and an increase in ventricular arrhythmias (5).

Numerous reports suggest that the endogenous opiate system may be involved in cardiovascular pathophysiology. The beneficial effect of antagonism of the endogenous opiates with naloxone suggests that this hormone system is activated in response to both hemorrhagic shock (6–8) and myocardial ischemia-reperfusion (9). Previous investigators reported that the endogenous opiate system regulates cardiovascular function via direct effects on the central nervous system (10, 11). However, more recent studies suggest that endogenous opiates may affect the heart directly,

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following hemorrhagic shock (6) and myocardial ischemia-reperfusion (9).

In the rat, BE is synthesized and released by the anterior pituitary (AP) and the neurointermediate lobe (NIL) of the pituitary (2). BE levels in the pituitary are affected by stress. In particular, the concentration of BE in the AP and the NIL are affected by immobilization stress (12). Although there is evidence of BE in cardiac tissue (1, 3), and stress has been documented to affect cardiac function (13, 14), the effect of stress on cardiac BE has not been determined.

It was the goal of the present investigation to determine if stress, either physical or cardiovascular, alters cardiac BE levels. This was accomplished by measuring BE and B-LPH in the hearts of male rats subjected to immobilization stress, hemorrhagic shock or pressure overload-induced cardiac hypertrophy.

Materials and Methods

Animals. Male Sprague Dawley rats (200–225 g) were obtained from the Buckshire Corporation (Perkasie, PA). Animals received food (Ziegler Diet; Buckshire Industries, Lansdale, PA) and water *ad libitum,* and were maintained at 22° ± 2°C on a 12:12-hr light:dark cycle (lights on at 07:00 hr).

Collection of the Heart. Immediately following sacrifice by decapitation, the heart was removed and placed in ice cold saline. The atria and remaining blood vessels were removed and an incision made in the septum and ventricular wall to expose the inner portion. The heart was washed in fresh cold saline, blotted dry and frozen immediately in liquid nitrogen.

Immobilization Stress. Physical stress was induced by immobilizing each animal in a Plexiglas restrainer and placing the animal on its back. After exposure to stress for either 30, 60, and 120 min, animals were sacrificed. Stressed animals were sacrificed alternately with nonstressed controls. Subsequent to sacrifice, the hearts were removed, washed, frozen in liquid nitrogen, and stored at −80°C.

Hemorrhage. Rats were anesthetized with pentobarbital sodium (40 mg/kg, ip) and a cannula (PE 250) was inserted into the trachea to maintain a patent airway. A polyethylene catheter (PE 50) filled with heparinized saline was inserted into the right carotid artery for the measurement of mean arterial blood pressure (MABP; measured with a Statham P23AC pressure transducer). The normal range for MABP of anesthetized rats is 100–130 mm Hg. A second polyethylene catheter was placed in the left femoral artery and was used for blood withdrawal. Animals were given heparin (500 IU/kg) prior to bleeding. Following a 30-min stabilization period, baseline hemodynamics were recorded and blood was withdrawn from the femoral artery as required to maintain a MABP of 45 mm Hg (average volume of 3.0 ± 0.1 ml/100 g body wt). The MABP was maintained at 45 mm Hg while the rat was reinfused with 20% of the maximum volume of blood withdrawn. At this time the remainder of the withdrawn blood was returned to the animal. Following reinfusion of the blood, the rats were observed until the MABP declined to 45 mm Hg (100 + 12 min) at which time they were sacrificed. No differences were found in the data obtained from anesthetized sham operated rats and anesthetized rats, thus data from both types of treatment were pooled for control values. Subsequent to sacrifice, the hearts were removed, washed, frozen in liquid nitrogen, and stored at −80°C.

Hypertrophy. Pressure overload cardiac hypertrophy was induced by abdominal aortic constriction. Under chloral hydrate anesthesia, the abdominal aorta was isolated above the renal arteries and the aortic lumen was narrowed using the ligature needle technique (15). Sham operated controls were subjected to all of the same surgical procedures except for aortic constriction. Following 4 weeks of aortic constriction, the animals were sacrificed. The hearts were collected, washed, frozen in liquid nitrogen, and stored at −80°C.

Extraction of β-Endorphin from Cardiac Tissue. The frozen heart was placed in 2.5 ml of 2 *N* acetic acid, boiled for 20 min. and cooled in an ice bath for 30 min. Each heart was homogenized and the resulting homogenate was centrifuged for 15 min at 10,000*g*. The supernatants obtained for three hearts were pooled, frozen, and lyophilized. The recovery of BE and B-LPH by this method ranged from 76%–86% (1).

Gel Chromatography. Lyophilized extracts of the heart rate were resuspended in 2 ml of chromatography buffer (0.1 *N* acetic acid, 0.05% bovine serum albumin and 0.02% sodium azide) and applied to a 0.9 × 45 cm glass column packed with Sephadex G-50 fine (Pharmacia Fine Pharmaceuticals, Piscataway, NJ). Fractions of 750 μl were collected and lyophilized. All lyophilized fractions were reconstituted with assay buffer and the immunoreactivity contained in each fraction was determined using the BE radioimmunoassay which recognizes BE and B-LPH. The elution profiles of the samples were compared with those obtained for camel BE and human B-LPH. Total amounts of BE and B-LPH were determined from the chromatograms (1).

Measurement of Beta-Endorphin by Radioimmunoassay (RIA). The details of the BE assay have been described previously (1, 12). Briefly, the antibody to BE (PR-69) was raised against human BE and demonstrated an 85% cross-reactivity with camel BE and 100% cross-reactivity with human B-LPH. The

lesser cross-reactivity of the antibody with camel BE suggests that the values obtained in the assay may actually be underestimates of the real values. The reference preparation and hormone for iodination was camel BE. The samples produced parallel displacement to the standard curve. The minimal detectable dose of the assay is 30 pg/tube and 50% displacement of the tracer occurs at approximately 300 pg/tube. All lyophilized fractions were reconstituted with assay buffer, and the immunoreactivity contained in each fraction was determined.

Statistical Analysis of the Data The data were analyzed using analysis of variance and Fischer's LSD, or the Student's t test. A significance level of $P < 0.05$ was used to establish significant differences.

Results

The Response of BE and B-LPH in the Heart to Immobilization Stress. As compared with prestress levels, 30 min of immobilization stress resulted in a significant rise ($P < 0.009$) in the content of BE in the heart and a significant decline ($P < 0.01$) in the content of B-LPH. These changes resulted in a significant increase ($P < 0.001$) in the ratio of BE:B-LPH in the cardiac tissue. Following 60 and 120 min of immobilization, BE levels in the heart returned to a value similar to that observed prior to immobilization. However, the content of B-LPH in the heart remained significantly diminished ($P < 0.01$) at 60 min and returned to the prestress value by 120 min. At 60 min of immobilization stress, the ratio of BE:B-LPH declined from that observed at 30 min, but still remained significantly greater ($P < 0.034$) than that observed in nonstressed controls. The ratio of BE:B-LPH in the heart returned to prestress levels by 120 min of exposure (Fig. 1).

The Effect of Hemorrhagic Shock on the Content of BE and B-LPH in the Heart. The content of BE in the heart was not affected by hemorrhagic shock. By contrast, the content of B-LPH in cardiac tissue was significantly reduced ($P < 0.005$) in animals subjected to hemorrhage as compared to controls. The result of the reduction of B-LPH was a significant elevation ($P < 0.025$) in the ratio of BE:B-LPH in the heart response to hemorrhagic shock (Fig. 2). An example of the chromatogram obtained is shown in Figure 3.

The Effect of Cardiac Hypertrophy on the Content of BE and B-LPH in the Heart. At 4 weeks following abdominal aortic banding, the heart weight: body weight ratio was significantly greater ($P < 0.001$) in banded versus sham-banded animals (3.69 ± 0.20 vs 2.68 ± 0.04), indicating the presence of cardiac hypertrophy. In order to account for the larger size of the hypertrophied hearts, BE and B-LPH were expressed per milligram wet weight. The concentration of BE in the heart was unchanged by hypertrophy, whereas the

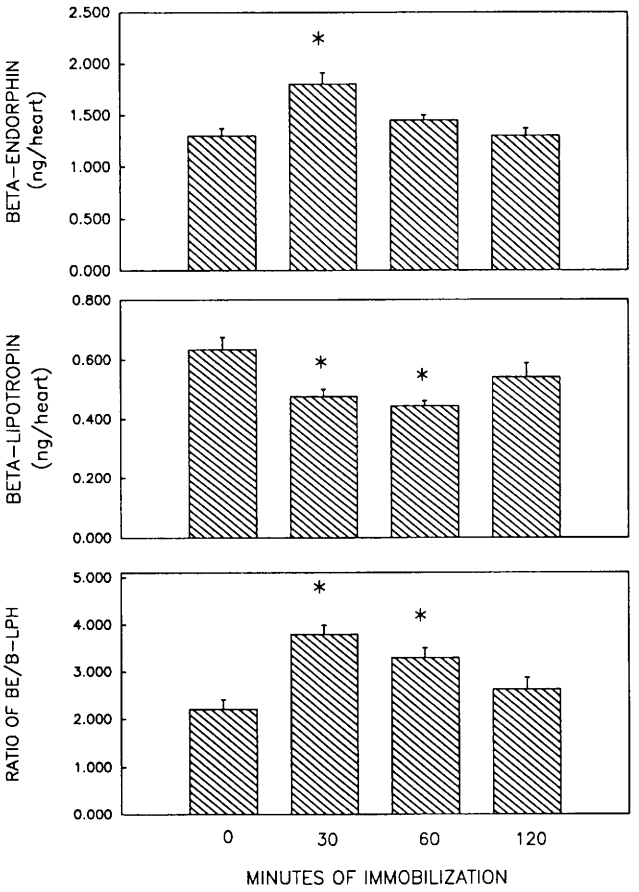

Figure 1. The content of BE (upper panel), B-LPH (middle panel), and the ratio of BE:B-LPH (lower panel) in cardiac tissue of male rats exposed to immobilization for varying periods of time. Each bar represents data obtained from four determinations of three pooled hearts, representing a total of 12 hearts. The error bars are ±one SEM; *$P < 0.05$.

concentration of B-LPH was significantly reduced ($P < 0.01$). The ratio of BE:B-LPH was significantly increased ($P < 0.001$) in hypertrophied as compared with control hearts (Fig. 4). An example of the chromatogram used to calculate the data is shown in Figure 5.

Discussion

The content of BE in the heart was increased in response to immobilization stress. Hemorrhagic shock resulted in a decrease in the content of B-LPH in the heart, while the content of BE was unaffected. Similarly, myocardial hypertrophy was associated with a decrease in the concentration of B-LPH in the heart, while the concentration of BE was unchanged. There was a significant increase in the ratio of BE:B-LPH in all three experiments. These findings suggest that BE levels in the heart are increased by physical stress (i.e., immobilization); however, cardiovascular stress (i.e., hemorrhage and hypertrophy) primarily affects levels of B-LPH.

BE is produced by direct cleavage from POMC

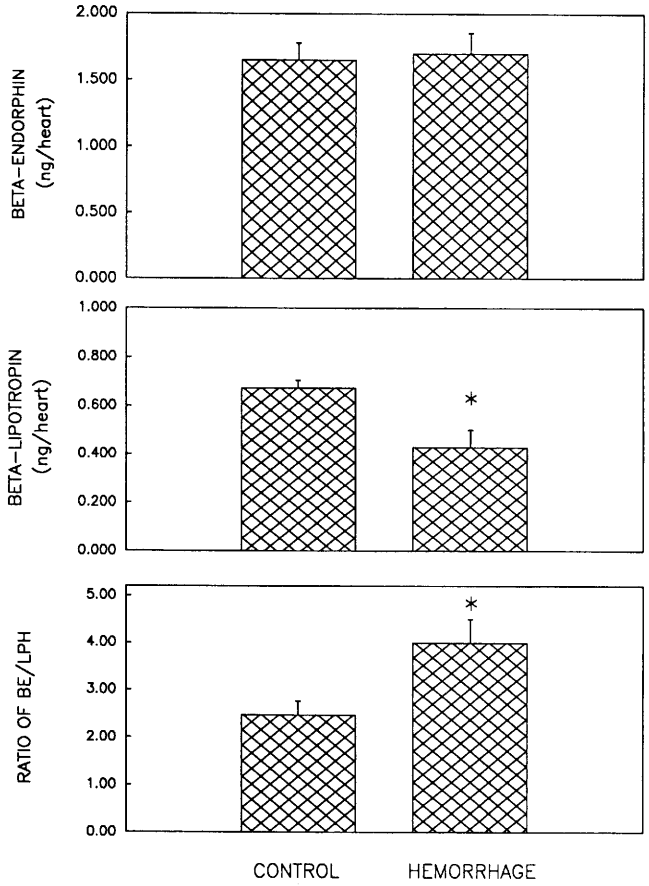

Figure 2. The content of BE (upper panel), B-LPH (middle panel), and the ratio of BE:B-LPH (lower panel) in cardiac tissue of male rats subjected to hemorrhagic and the corresponding controls. Each bar represents data obtained from four determinations of three pooled hearts, representing a total of 12 hearts. The error bars are ±one SEM; $*P < 0.05$.

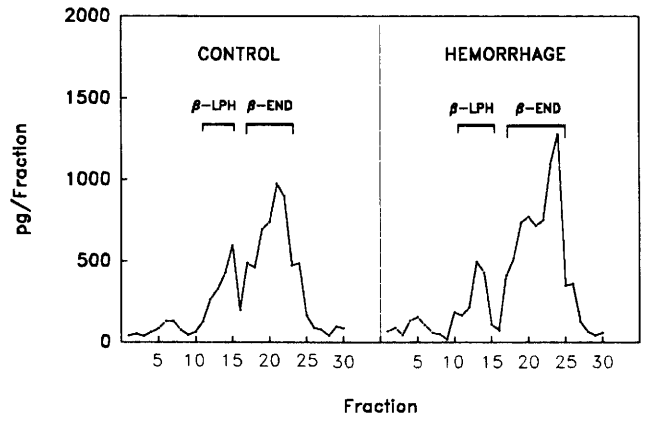

Figure 3. Chromatogram of β-endorphin (B-END) and β-lipotropin (B-LPH) obtained from three hemorrhaged and three control hearts.

and by cleavage from B-LPH (2). The cleavage of BE from POMC is accomplished by the prohormone convertase, PC2 (16–18). When BE is cleaved from POMC directly, no B-LPH is generated. The cleavage of B-LPH from POMC occurs via the prohormone convertase, PC1, which also appears to cleave a minimal

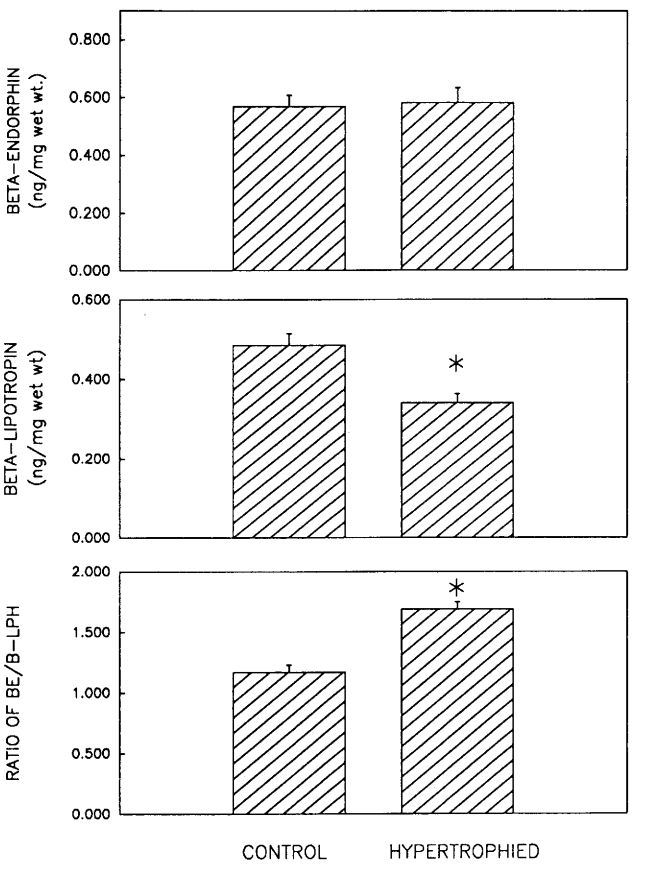

Figure 4. The concentration of BE (upper panel), B-LPH (middle panel), and the ratio of BE:B-LPH (lower panel) in hypertrophied cardiac tissue of male rats. Each bar represents data obtained from four determinations of three pooled hearts, representing a total of 12 hearts. The error bars are ± one SEM; $*P < 0.05$.

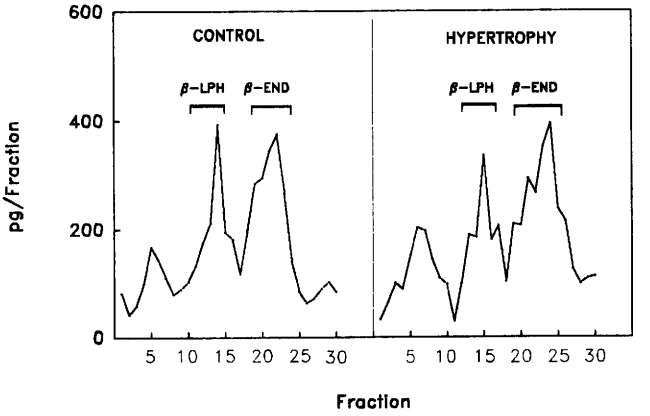

Figure 5. Chromatogram of β-endorphin (B-END) and β-lipotropin (B-LPH) obtained from three hypertrophied and three control hearts.

amount of BE from B-LPH (16–18). The cleavage of BE from B-LPH is highly promoted by PC2 (16–18). Several investigations have suggested that the majority of BE results from the PC2-induced cleavage of POMC rather than PC1 or PC2 directed cleavage of B-LPH (16–18).

The observed effects of immobilization stress on BE and B-LPH in the heart may be speculated to be

the result of an increase in PC2 activity. Increased synthesis of BE by cleavage of POMC would increase the content of BE without altering the content of B-LPH. Increased PC2 activity would also promote cleavage of BE from B-LPH thereby increasing BE and decreasing B-LPH. The final result of an increase in PC2 activity would be an increase in BE, a decrease in B-LPH and an increase in the ratio of BE:B-LPH, all of which were observed following immobilization stress. A previous report indicates that increased PC2 activity results in an increase of BE, a decrease of B-LPH, and an increase in the ratio of BE:B-LPH (18). Alternatively, an increase of BE and a decrease of B-LPH may reflect a decrease in the metabolism of BE and an increase in the degradation of B-LPH. Although description of mechanisms is purely speculative, it is clear that immobilization stress increases the amount of BE in the heart and results in a decrease of B-LPH.

Several types of physical stress have been reported to affect cardiovascular function in the rat. Intermittent tailshock increases heart rate, mean arterial pressure, total peripheral resistance and cardiac index (13). Immobilization stress results in an increase in systolic pressure, diastolic pressure and peripheral resistance (14). It is not clear, however, whether these responses reflect a central effect, a peripheral effect, or both. Evidence indicates that BE is synthesized in the heart (3) and, in the present study, the content of BE in the heart was increased by immobilization stress. It is possible that the synthesis of BE is increased in order to diminish or partially offset the stimulation of heart function produced by physical stress.

Hemorrhage and hypertrophy both resulted in a decrease of B-LPH with no change in BE. These results may reflect a stability of the degree of cleavage of BE from POMC by PC2 and a decrease in the degree of cleavage of B-LPH from POMC by PC1. Since the majority of BE is thought to result from the cleavage of POMC rather than B-LPH (16–18), a reduction in PC1 activity and no change in the activity of PC2, may result in a constant level of BE, a decrease in B-LPH, and an increase in the ratio of BE:B-LPH.

An alternative explanation for the lack of change in the level of BE in response to cardiovascular stress is that PC1 activity and possibly the activity of PC2 was increased. Excess BE may be produced and subsequently released into the interstitium, where it was bound by opiate receptors and rapidly degraded. This would result in a decrease in B-LPH with no overall change in BE. The data also may be interpreted to suggest that BE synthesis and release are unchanged and that either the synthesis of B-LPH is diminished or its degradation increased. Although there is no evidence to our knowledge that B-LPH modulates cardiovascular function, a reduction of B-LPH may indicate a compensatory mechanism in the heart for regulating its function which is separate from the effects of BE. Regardless of specific mechanism, these data indicate that the heart responds to cardiovascular stress by selectively altering the processing of POMC and subsequently the amount of B-LPH and the amount of BE relative to B-LPH. These observations provide evidence suggesting that BE, and possibly B-LPH, may participate in the regulation of heart function.

There are indications that opiates have a direct effect on the heart following hemorrhagic shock (6). In the dog, direct administration of naloxone into the coronary circulation significantly reversed reductions in arterial pressure, myocardial contractility and cardiac output resulting from the induction of hemorrhagic shock (6). Our data suggest opiate involvement in the heart following hemorrhagic shock since B-LPH levels in the heart and the amount of BE relative to B-LPH were altered.

The cardiomyopathic hamster is a model for cardiac hypertrophy and the effects of congestive heart failure. It has been reported that in cardiomyopathic hamsters, there is a 3- to 4-fold increase in mRNA for preproenkephalin A, the precursor for the enkephalin family of endogenous opioid peptides (19). It may be speculated that alterations in the synthesis of BE and B-LPH, and an increase in the enkephalin precursor message, represent specific changes in the activity of the different families of endogenous opiates, in response to cardiac hypertrophy in rodents.

The effect of immobilization stress on cardiac BE was transient and differed from the effects of hemorrhage and hypertrophy. The animals appeared to acclimate to the effects of acute physical stress. Unlike immobilization, hemorrhage and hypertrophy are specific to the cardiovascular system. It was not possible to determine if the effect of hemorrhage on the heart was transient. However, since hypertrophy represented a chronic alteration in cardiac activity, the results obtained could not be considered as short-lived. At this time, no generalizations can be made as to if all forms of cardiovascular challenge will have the same influence on the levels of POMC derivatives in the heart. At least in the present study, two different forms of cardiovascular manipulation did produce the same effect.

The occurrence of endogenous opiates in cardiac tissue has been reported by several laboratories. BE (1, 20), and the mRNA for the prohormone from which BE is derived (3), have been identified in the rodent heart. Enkephalins (21, 22) and dynorphin (23) have been demonstrated in the hearts of rodents, and the mRNA for the enkephalin prohormone is also found to be present (24). It appears that endogenous opiates can also be released from the human heart. BE, met-

enkephalin, leu-enkephalin, and dynorphin have been detected in human coronary sinus blood (25), and BE has been observed in the pericardial fluid (26). In addition, BE in the coronary sinus blood tended to be increased by epidural spinal electrical stimulation in patients with coronary artery disease in which angina was induced by atrial pacing (25). These findings indicate that endogenous opioid peptides may serve a paracrine role in the regulation of the heart.

The observations made in the present investigation indicate that when presented with a challenge to the cardiovascular system, the heart may respond by altering the intracardiac synthesis of BE and the amount of BE relative to B-LPH. The significance of changes in POMC-related peptides in response to stress, hemorrhage, and pressure overload may be that locally synthesized BE, B-LPH, or both may modulate the functional activity of the heart, suggesting a paracrine function for one or both of these hormones.

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