Localization of β-Endorphin in the Rat Heart and Modulation by Testosterone[1] (42855)

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Abstract. **Chromatographic analysis and radioimmunoassay were used to identify and quantitate β-endorphin (BE) and β-lipotropin (B-LPH) in the hearts (devoid of major blood vessels and atria) from intact male rats, castrated male rats, and castrated male rats treated with testosterone proprionate (TP). BE and B-LPH in the plasma of these animals were also identified and measured. In comparison to intact animals, castration resulted in a significant elevation in the content of BE in the heart which was reversed by the administration of TP. The content of B-LPH in the heart was not affected by castration or castration in combination with TP. The ratio of BE to B-LPH in the heart of castrated animals was significantly elevated as compared with intact controls. Treatment of castrates with TP returned the ratio of BE to B-LPH to that observed in intact animals. The concentration of BE in the plasma was greater in castrated rats and castrated rats given TP than in intact males, whereas the concentration of B-LPH was diminished in castrated animals given TP. The ratio of BE to B-LPH was greater in castrated animals treated with TP than in castrated and intact animals. The content of BE and B-LPH, as well as the ratios of the two peptides, varied independently in the cardiac tissue and plasma. The present findings indicated that (i) BE and B-LPH are present in cardiac tissue, (ii) the amount of BE and B-LPH in the heart and the ratio of BE to B-LPH appear to be modulated by TP, and (iii) BE and B-LPH detected in the heart was not simply a reflection of the presence of these peptides in the plasma.** [P.S.E.B.M. 1989, Vol 190]

The endogenous opioid peptide, β-endorphin (BE), is present in both the rat pituitary and hypothalamus (1, 2). BE has also been localized in peripheral tissues in the rat. For example, the kidney (3), gastric antrum (4), pancreas (5), and the perikarya and nerve fibers of the myenteric and submucosal plexus of the duodenum (6) have all been shown to contain BE. BE is also found in the testes (3, 7, 8), prostate (7), seminal vesicle (7, 8), vas deferens and epididymus (8) of the male rat, and the ovaries (9, 10) of the female rat. The presence of BE precursor messenger RNA in peripheral sites containing BE (3, 11–14) suggests that it is synthesized by these organs and may serve a paracrine function.

The endogenous opioid system is acknowledged to participate in the physiologic regulation of cardiovascular function via an action on the central nervous system (15–17). Recent evidence suggests however, that endogenous opiates may also exert a direct effect on the heart (18, 19). The concept of a direct regulation of the heart by endogenous opiates is supported by the presence of methionine and leucine enkephalin (20, 21) and dynorphin (22) in the rodent heart, primarily in ganglia and paraganglionic cells (22).

In male rats, testosterone appears to affect the content and concentration of BE in the hypothalamus (23–25) and to modulate the effects of acute and chronic stress on the concentration of BE in the pituitary (24). Regulation of the content of BE in the pituitary and hypothalamus by the neurotransmitter dopamine, and BE content in the pituitary by norepinephrine, has also been observed to be modulated by testosterone (Forman and Estilow, unpublished data). These observations suggest that in male rats, the content and concentration of BE are influenced by the gonadal steroid environment.

The present study was conducted to determine if BE and β-lipotropin (B-LPH), the products of the enzymatic cleavage of proopiomelanocortin (26), could be localized in the heart. Once the presence of BE and B-LPH in the heart was indicated, the study proceeded to determine if their occurrence was affected by testosterone.

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Materials and Methods

Animals. Sprague-Dawley male rats (225–250 g) were obtained from Ace Animals, Inc., Boyertown, PA. Animals were maintained in a temperature-controlled room (22 ± 2°C) and kept on a 12:12 light:dark cycle (lights on at 0700 hr). Food (Ziegler Diet, Buckshire Farms, Landsdale, PA) and water were available *ad libitum*.

Gonadal Steroids. Testosterone proprionate (TP; Sigma Chemical Co., St Louis, MO) was suspended in corn oil and administered subcutaneously at a dose of 500 μg/kg/day. This dose should compensate for the absence of the testes (24, 27). Controls received the corn oil vehicle alone.

Castration and Pretreatment with Gonadal Steroids. Rats were surgically castrated under ether anesthesia. After 1 week of recovery, one half of the animals were administered daily injections of TP and the remaining animals were given daily injections of corn oil. The respective treatments were continued for 4 weeks. Animals were killed at the end of the 4-week period.

Collection of Plasma. Animals were decapitated and blood was collected into 600 μl of EDTA in sterile saline. The blood was centrifuged at 1000*g* for 10 min at 4°C. The resulting plasma was removed and stored at −80°C until assayed for β-endorphin and β-lipotropin.

Collection of the Heart. Immediately following decapitation the heart was removed and immersed in ice-cold saline. The heart was then placed on gauze and the remainder of the major blood vessels and atria were removed. A cut was made in the septum and ventricular wall of the heart to expose the interior portion. The heart was then immersed for a second time in ice-cold saline, after which it was blotted dry and frozen in liquid nitrogen. The frozen tissue was stored at −80°C.

Extraction of 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 then homogenized. The resulting homogenate was centifuged for 15 min at 10,000*g*. The supernatants obtained from three hearts were pooled, frozen, and lyophilized. The recovery of BE and B-LPH by this method ranged from 76 to 86% ($n = 3$ determinations for each peptide).

Gel Chromatography. Lyophilized extracts of the heart were resuspended in 1 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- × 40-cm glass column packed with Sephadex G-50 fine (Pharmacia Fine Pharmaceuticals, Piscataway, NJ). Fractions of 750 μl were collected and lyophilized. Plasma samples were chromatographed in a 1.6- × 70-cm glass column packed with Sephadex G-50 fine. Three milliliters of pooled plasma were applied to the column and fractions of 1.5 ml were collected and lyophilized (24, 28). All lyophilized fractions were reconstituted with assay buffer and the immunoreactivity contained in each fraction was determined using the β-endorphin radioimmunoassay which recognized β-endorphin and β-lipotropin. The elution profiles of the samples were compared with those obtained for either camel β-endorphin or human β-lipotropin. The portion of the elution profile corresponding to β-endorphin may have also included its C terminally shortened fragments and acetylated forms, which are also recognized by the antibody to β-endorphin. Chromatographic processing of the standards for camel β-endorphin and human β-lipotropin did not result in the appearance of multiple peaks. Total amounts of β-endorphin and β-lipotropin were determined from the chromatograms.

Radioimmunoassay of Immunoreactive β-Endorphin. Immunoreactive BE was measured by a double antibody radioimmunoassay as described previously (24, 29, 30). In summary, the antibody to β-endorphin was raised against human β-endorphin$_{1-31}$ (the generous gift of Dr. C. H. Li, University of California at San Francisco). On a molar basis, the antibody to human β-endorphin cross-reacted 85% with camel β-endorphin$_{1-31}$ and 100% with human β-lipotropin (generously supplied by the National Pituitary Agency) and β-endorphin fragments, β-endorphin$_{1-27}$, and *n*-acetyl β-endorphin$_{1-27}$. No cross-reactivity with γ-endorphin, met-enkephalin, leu-enkephalin, α-melanocyte stimulating hormone, or adrenocorticotropin was determined at doses up to 10 ng/tube. The reference preparation and hormone for iodination was camel β-endorphin$_{1-31}$ (Peninsula Laboratories, San Carlos, CA). Camel β-endorphin was iodinated using the chloramine T method. The sample volumes used produced parallel displacement to the standard curve. The minimal detectable dose of the assay was 30 pg/tube and 50% displacement of the tracer occurred at a dose of approximately 300 pg/tube. The intra- and interassay coefficients of variation were 7 and 12%, respectively.

Statistical Analysis. Statistical analysis of the data was performed using a two-way or three-way analysis of variance. Subsequent testing was done using the Newman-Keul procedure. Resulting probabilities of $P < 0.05$ were accepted as significantly different.

Results

BE and B-LPH in the Heart. As compared with intact animals, the content of BE in the heart was significantly elevated in response to castration. Treatment with TP diminished the content of BE in the heart of castrated males to that observed in intact animals. The content of B-LPH in the heart was similar in intact animals, castrated animals, and castrated animals given TP. The ratio of BE to B-LPH in the heart was significantly greater in castrated males than in intact animals. Administration of TP appeared to reverse the effect of castration on the ratio of BE to B-

LPH in the heart (Table I). An example of the chromatographic profiles obtained from the acid extracts of hearts (ventricular tissue) from intact male rats, castrated male rats, and castrated male rats treated with TP is shown in Figure 1. The presence of multiple peaks in the region considered to be BE may represent smaller molecular weight fragments of BE recognized by the β-endorphin antibody.

BE and B-LPH in the Plasma. The concentration of BE in the plasma was lower in intact animals as compared with castrated male rats and castrated male rats administered TP. The concentration of B-LPH in the plasma was the same in intact and castrated animals and was diminished in castrated animals by the administration of TP. The ratio of BE to B-LPH was greater in castrated male rats given TP than in intact and castrated animals (Table I). Representative chromatograms for BE and B-LPH in the plasma of intact male rats, castrated male rats, and castrated male rats administered TP are shown in Figure 2. The occurrence of several peaks in the region of the chromatogram considered to be BE may be attributed to the recognition by the antibody of smaller molecular weight fragments of BE. The presence of a second peak of B-LPH in the plasma of intact animals is believed to be an artifact since we have not observed multiple peaks of B-LPH in other chromatograms obtained in this or other studies. Furthermore, repeated chromatographic analysis of the B-LPH standard did not result in the occurrence of more than one peak of immunoreactivity.

Comparison of BE and B-LPH in the Heart and Plasma. The content of BE in the heart was consistently greater than that observed in the plasma. The amount of B-LPH in the two compartments appeared to be

Table I. BE and B-LPH in the Heart and Plasma of Castrated Male Rats, Castrated Male Rats Administered TP, and Intact Male Rats[a,b]

	Heart		
	Intact	Castrated	Castrated + TP
BE (pg/heart)	552 ± 33	1,914 ± 157[c,d]	606 ± 55
B-LPH (pg/heart)	474 ± 39	402 ± 34	436 ± 72
Ratio of BE:B-LPH	1.16 ± 0.05	4.76 ± 0.45[c,d]	1.39 ± 0.25
	Plasma		
	Intact	Castrated	Castrated + TP
BE (pg/ml)	268 ± 25	369 ± 27[c]	425 ± 33[c]
B-LPH (pg/ml)	558 ± 57	675 ± 44	416 ± 20[c,e]
Ratio of BE:B-LPH	0.51 ± 0.06	0.55 ± 0.06	1.02 ± 0.12[c,e]

[a] Each value represents a mean ± 1 SEM.
[b] Values were obtained from a total of 12 animals.
[c] Differs significantly from intact male rats.
[d] Differs significantly from castrated male rats treated with TP.
[e] Differs significantly from castrated male rats.

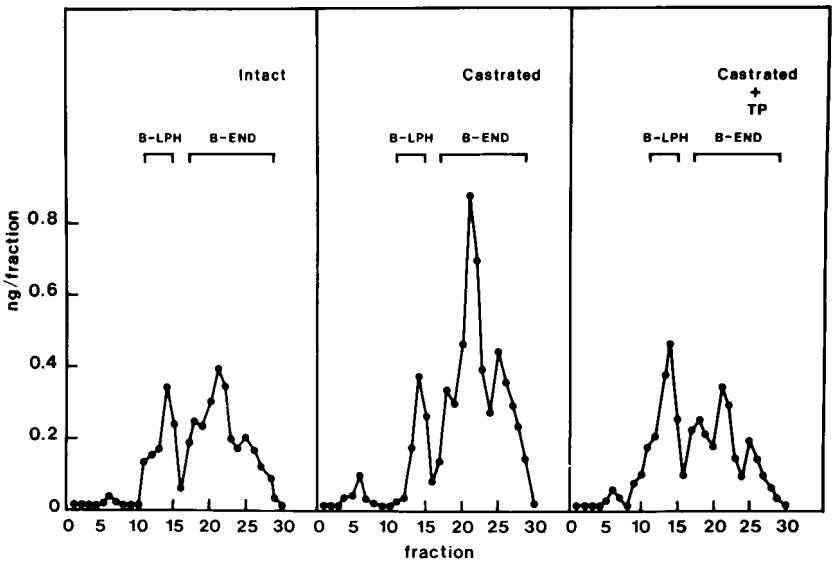

Figure 1. Chromatographic profiles of extracts from the hearts of intact (left panel), castrated (center panel), and castrated TP-treated (right panel) male rats. The portions of the profile considered to be BE and B-LPH are indicated.

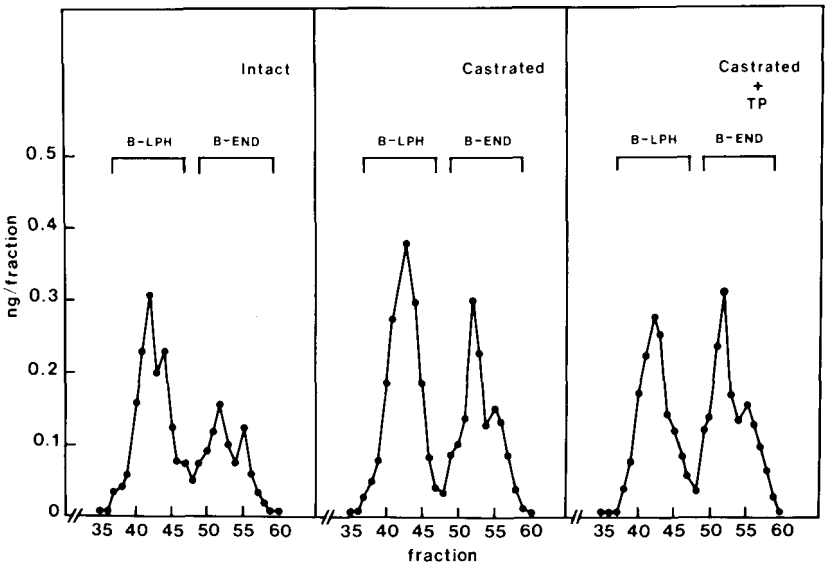

Figure 2. Chromatographic profiles of plasma from intact (left panel), castrated (center panel), and castrated TP-treated (right panel) male rats. The portions of the profile considered to be BE and B-LPH are indicated.

similar in intact animals and castrated animals given TP. In castrated males, the amount of B-LPH was greater in the plasma than in the heart. The ratios of BE to B-LPH in the heart were greater in intact and castrated male rats and similar in castrated male rats treated with TP. Castration and castration plus gonadal steroid administration did not produce a similar pattern of change in BE, B-LPH, and the ratio of BE to B-LPH in the heart and plasma.

Discussion

The results of this study indicate that detectable amounts of BE and B-LPH occur in rat heart (ventricular tissue) and that the content of BE in cardiac tissue appears to be modulated by the gonadal steroid, testosterone. The content of BE and B-LPH in the heart does not appear to be a direct reflection of BE and B-LPH in the plasma since changes in the content of BE and B-LPH in the heart and the concentration of BE and B-LPH in the plasma, as well as the ratios of BE to B-LPH in these two compartments, were not observed to correspond.

BE has been detected in numerous organs in the periphery of the rat (3, 4, 7–10). BE detected in these tissues may have resulted from the binding of BE circulating in the plasma. Opiate receptors in the liver, kidney, spleen, testes, mammary gland, and uterus support such a conclusion (31). In many tissues, however (i.e., testes (3, 12, 14), ovaries (11, 13), kidneys (3)), the occurrence of BE may result from synthesis *in situ* as suggested by measurable amounts of messenger RNA for proopiomelanocortin, the precursor of BE and B-LPH (26). A gonadal steroid-mediated synthesis of opioid peptides in the heart has been postulated based on the identification of proenkephalin messenger RNA, the production of which is influenced by castration and gonadal steroid treatment (32). In the present study, differences were observed in the ability of castration and gonadal steroid replacement to affect the content of BE in the heart and the concentration of BE in plasma. In intact animals, the ratios of BE to B-LPH in the heart and plasma were found to exhibit a 2-fold difference, and in response to castration, the difference approached almost 10-fold. Only in castrated animals treated with TP were the ratios of BE to B-LPH in the heart and plasma found to be the same. These data suggest that whatever the mechanism responsible for the presence of BE in the heart, it is modulated by gonadal steroid.

Castration and gonadal steroid replacement clearly influenced the content of BE in the heart and the ratio of BE to B-LPH. In the rat, gonadal steroids have been found to influence the amount of BE in several tissues which synthesize BE directly. The content (23, 25) and concentration (24) of BE levels in the hypothalamus are diminished by testosterone in male rats. In female rats estrogen decreases the amount of BE in the hypothalamus (28, 29, 33, 34), apparently by reducing the relative amount of messenger RNA coding for its prohormone, proopiomelanocortin (35). Gonadal steroids also influence the amount of BE in the pituitary gland of male (24, 36) and female (29, 30, 36, 37) rats, and our laboratory has recently observed that in both male (24) and female (30) rats, gonadal steroids modulate the effects of stress, in the form of immobilization, on the concentration of BE in the pituitary. Much like these systems, it appears that BE in the heart is selectively modulated by the circulating gonadal steroid environment.

Several lines of evidence indicate the activity of the heart may be affected directly by endogenous opiates. Using an isolated Langendorff heart preparation, administration of morphine, leucine enkephalin, and methionine enkephalin reduced coronary pressure, decreased heart rate, and lowered developed tension (19). In intact animals, direct infusion to the heart of the opiate antagonist, naloxone, increased left ventricular contractile force, left coronary blood flow, and myocardial oxygen consumption. Injection of naloxone into the right atrium was not followed by similar effects, mitigating against a systemic mechanism, and the rapid onset of these effects were interpreted to suggest the presence of endogenous opiates operating locally in the myocardium (18). The presence of BE may further indicate the occurrence of an intrinsic opiate system in the heart which participates in the regulation of coronary function.

By the methods employed, BE and B-LPH were found to occur in measurable amounts in ventricular tissue of the rodent heart. The content of BE in the heart was observed to be influenced by castration and testosterone. The origin of the BE and B-LPH detected in cardiac tissue is not clear at the present time; however, it does appear that the presence of these peptides in the heart is not a simple reflection of their occurrence in the plasma. These data add support to the hypothesis of opioid regulation of cardiovascular function by a direct action on the heart.

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Erratum

The article Molecular Cloning of the Complementary DNA for a Human Folate Binding Protein by Sadasivan and Rothenberg which appeared in the November 1988 issue contained an error on page 243, line 17, right column.

The sentence reads:

> The similar size of each insert may explain why four of the six clones (rather than the expected two of six clones) . . .

It should read:

> The similar size of each insert may explain why four of the six clones (rather than the expected *one* of the six clones) . . .