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β-ENDORPHIN IN MALE RAT REPRODUCTIVE ORGANS

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SUMMARY

β-Endorphin and related peptides have been detected within the male rat reproductive system. The β-endorphin immunoreactivity of testis exhibits parallelism with synthetic human β-endorphin on serial dilution and coelutes with β-endorphin on Sephadex G-50 and reverse phase high pressure liquid chroma tography. Sephadex G-50 chromatography also indicates the presence of β-lipotropin. β-Endorphin concentrations in rat seminal vesicles and prostate were approximately 25% of that in testis.

β-Endorphin and other opioid peptides have been identified in the brain, pituitary and gastrointestinal tract by radioimmunoassay, bioassay and immunohistochemistry (1-9). This widespread distribution and the presence of opioid receptors in the mouse vas deferens (10) prompted us to look for opioid peptides in the male rat reproductive system. We report here evidence for both β-endorphin and β-endorphin-like immunoreactive peptides in extracts of testis, prostate and seminal vesicles from sexually mature rats.

MATERIALS AND METHODS

200 g male Sprague-Dawley rats given access ad libitum to water and standard rat chow were decapitated and the following reproductive organs were immediately removed: a single testis (decorticate), single caudate epididymis, prostate, seminal vesicles and both vas deferens. Each was weighed and placed into 1.0 ml of 0.2 M HCl at 97°C for 15 minutes to destroy enzyme activity (3,11). Samples were homogenized with a Polytron for 5-10 seconds. The residue on the homogenizer probe was recovered by rinsing it into the corresponding homogenate with 1.0 ml of HCl. Homogenates were centrifuged at 1,000 x g for 1 hour, and then the supernatants were aspirated and frozen at -20°C. After thawing at 26°C, the pH of each sample was adjusted to 7.5 with 1.0 N NaOH and then the sample was frozen at -20°C. After thawing the sample, a final centrifugation at 1,000x g yielded a clear, colorless supernatant for chromatography and RIA.

A pooled testis extract, prepared from equal volumes of the supernatant from 8 rats, was chromatographed on a 0.9x60 cm column of Sephadex G-50 precoated with 2% crystalline bovine serum albumin and eluted with 0.2 M acetic acid, 0.01% Triton-X-100. Fractions of 0.62 ml were collected at 0.15 ml/minute. Prior to radioimmunoassay (RIA), each fraction was raised to pH 7.5 with 1.0 N NaOH.

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Reversed phase high pressure liquid chromatography was performed with an Altex system interfaced to a computerized gradient making accessory. A flow rate of 1.0 ml/min was maintained through an Altex ODS Ultrasphere column, 4.6 mm x 25 cm, 5 μm particle size, with a backpressure of less than 2,000 psi. Linear acetonitrile (Fisher Scientific Co.) gradients with stepwise increasing slopes were used (modified from Ref. 12). The sample and starting buffer consisted of 0.1 M phosphate, pH 2.1. Two and one-half min after sample injection a linear acetonitrile gradient was begun which rose to 40% at 37.5 min after injection. From this point the acetonitrile increased linearly to 60% at 45 min and to 100% at 55 min after sample injection. Retention times for methionine enkephalin, leucine enkephalin and human β-endorphin were measured by injecting 100 μg/ml of each of the purified peptides and detecting the ultraviolet absorbance at 225 nm. Fractions from the G-50 chromatography of the testis extract, which demonstrated β-endorphin immunoreactivity eluting in the region of synthetic human β-endorphin, were combined, lyophilized and reconstituted in starting buffer. A 250 μl sample was injected into the system, and fractions were collected at 1 min intervals. Fractions of interest were raised to pH 7.5, lyophilized and reconstituted in 0.45 ml of RIA buffer.

β-Endorphin was quantified by an RIA (13) which showed equimolar cross-reactivity with purified β-lipotropin (NIAMDD) and no displacement by α and γ-endorphin (Peninsula Lab), methionine and leucine enkephalin (Calbiochem.), or ACTH (1-24). The minimum detectable dose was 2.2 pg/tube (22 pg/ml of unknown) and the intra-assay coefficient of variation using human β-endorphin (obtained from NIAMDD) was 4%. 0.05 M phosphate, pH 7.7, containing 0.75% crystalline bovine serum albumin and 0.01% merthiolate was used as the RIA buffer. [^{125}I]-β-endorphin with a specific activity of 125-175 μCi/μg was prepared using sodium iodide (Amersham) according to the chloramine-T method (14). [^{125}I]-β-endorphin and [^{125}I]-iodide were separated using a 10 ml G-50 column eluted with 0.2 M acetic acid and 0.1% Triton-X-100. Unknown samples of 100 μl were added to 200 μl of rabbit anti-β-endorphin antibody (Bioflex Corp) diluted 1/8000 in RIA buffer. After 40 hours of incubation at 4°C, [^{125}I]-β-endorphin was added, and separation was begun 14 hours later by adding normal rabbit serum and goat anti-rabbit γ-globulin. The doses of the standard curve and unknown samples were prepared in triplicate.

RESULTS AND DISCUSSION

Serial dilution of pooled testis extract from 8 rats showed β-endorphin immunoreactivity which was parallel to the RIA standard curve (Fig 1) (statistical analysis, Ref. 15). Sephadex gel chromatography of this pooled specimen demonstrated β-endorphin immunoreactivity corresponding to the regions where β-lipotropin (detected by radioimmunoassay) and [^{125}I]-β-endorphin (Fig 2) elute. The molar ratio of β-endorphin/β-lipotropin was 6.1. In addition a small peak was detected at the void volume which could represent the 31,000 dalton precursor to β-lipotropin and β-endorphin.

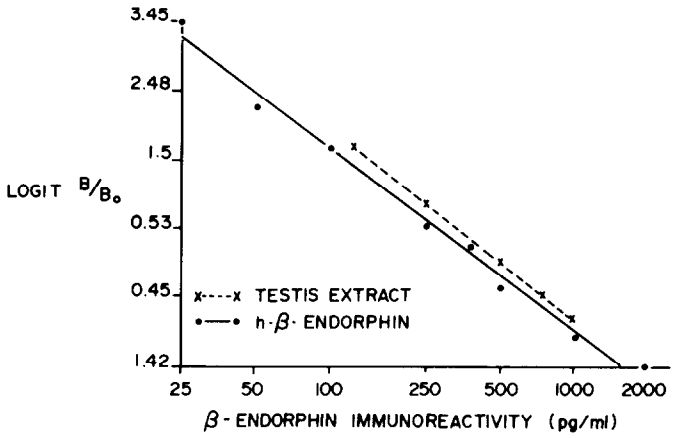

Fig. 1. Serial dilution of synthetic human β-endorphin detected by radioimmunoassay compared with serial dilution of rat testis extract.

High pressure liquid chromatography of the G-50 fractions corresponding to the β-endorphin region confirmed the presence of β-endorphin (Fig 3). The broadening of the elution profile about the fraction where synthetic human β-endorphin elutes may represent the micro-heterogeneity of β-endorphin

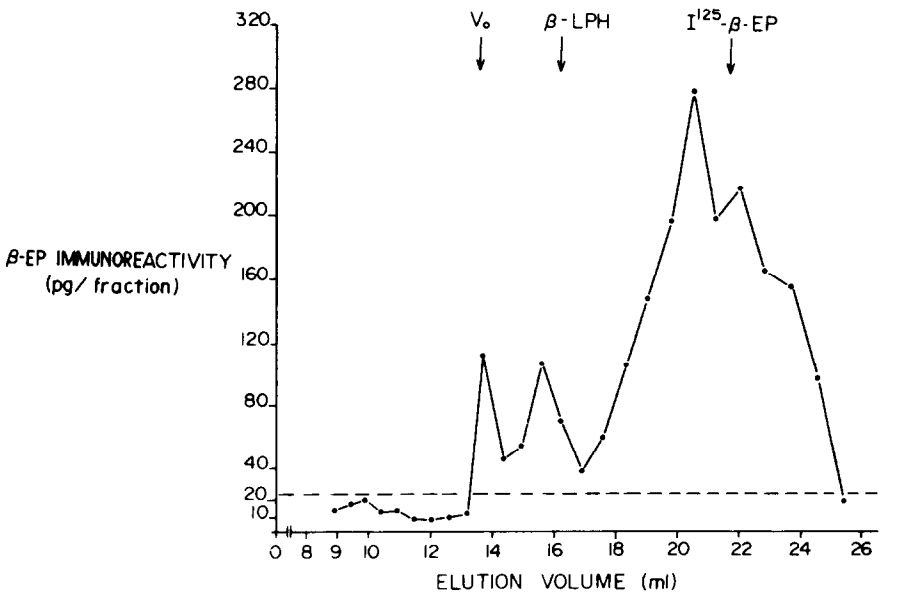

Fig. 2. Sephadex G-50 chromatography of rat testis extract detected by radioimmunoassay. --- indicates the minimum detectable concentration of human β-endorphin.

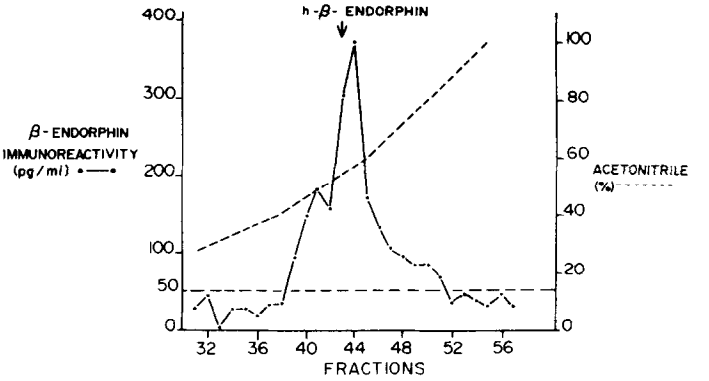

Fig. 3. High pressure liquid chromatography of rat testis extract obtained from [I^{125}]-β-endorphin region of Sephadex G-50 chromatography. — — — indicates minimum detectable concentration of human β-endorphin.

due to variation in the native chemical forms of tissue endorphin (16), extraction effects, or both. However, we found only a single, narrow elution peak with constant width at half height of 0.5 minute before and after heating synthetic β-endorphin to 97°C for 15 minutes in 0.2 M HCl. Considered together, the parallelism and chromatographic profiles provide strong evidence for the presence of β-endorphin and other immunoreactive peptides such as β-lipotropin in the rat testis. In addition, since the reported levels for β-endorphin immunoreactivity in basal rat serum are between 200-400 pg/ml (17,18), the amount of 1,250 pg/g in rat testis (Table 1) significantly exceeds that which might occur due to blood contamination of the extract. Data for β-endorphin immunoreactivity in the

Table 1
β-Endorphin Immunoreactivity in the Organs of the Rat Reproductive System (mean ±SD)

Tissue	N	β-endorphin immunoreactivity (pg/mg)
Testis	7	1.25 ±0.15
Prostate	4	0.28 ± .04
Seminal Ves.	4	0.24 ± .09
Epididymis	4	0.0
Vas deferens	4	0.0

testis was not corrected for extraction efficiency which was found to be 85% following the addition of 1.0 ng synthetic human β-endorphin to each of 4 testes prior to homogenization.

To diminish the effect of the large differences in weight between the testis and other reproductive organs, 2 extracts from each type of organ were pooled and concentrated at least 5-fold. β-Endorphin immunoreactivity which serially dilutes in parallel to the standard curve was found in the seminal vesicles (data not shown). Although the response of the prostate extract was not parallel to the β-endorphin standard, this may reflect the increased response variance at the low dose region of the standard curve. The tissue levels for both prostate and seminal vesicles were approximately 25% of those in the testis (Table 1). The inability to detect β-endorphin immunoreactivity in vas deferens and epididymis may reflect the very small amounts of tissue used for extraction. As an extra-reproductive control, mesenteric fat (N=4) was examined; β-endorphin was not detected.

β-Endorphin immunoreactivity is present in testis at levels which are consistent with binding by testicular receptors or de novo synthesis. This suggests that β-endorphin and other opioid peptides may have important regulatory functions in the reproductive system.

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