Pharmacology Biochemistry and Behavior, Vol. 60, No. 3, pp. 709–718, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00045-8

Genetic Relationship Between Central β-Endorphin and Novelty-Induced Locomotor Activity

RICHARD A. RADCLIFFE* AND V. GENE ERWIN†

*Institute for Behavioral Genetics, University of Colorado, Campus Box 447, Boulder, CO 80303
†School of Pharmacy, University of Colorado Health Sciences Center, Campus Box C238, 4200 East 9th Avenue, Denver, CO 80262

Received 8 August 1997; Revised 18 December 1997; Accepted 30 December 1997

RADCLIFFE, R. A. AND V. G. ERWIN. *Genetic relationship between central β-endorphin and novelty-induced locomotor activity*. PHARMACOL BIOCHEM BEHAV **60**(3) 709–718, 1998.—The goal of research presented in this report was to investigate the possibility that differences in β-ED levels could account for some portion of genetically mediated variation in locomotor activity. Subjects representing six of the LSXSS RI mouse strains were activity tested in a novel environment. Significant effects of strain and time as well as a significant strain by time interaction were detected. β-ED levels were estimated from several brain regions and the pituitary of naive and activity-tested mice. Significant strain effects were detected in all brain regions but not in the pituitary. There was no overall effect of exposure to novelty, but some strains showed either a decrease or an increase in β-ED levels in the septum, amygdala, and midbrain. A significant genetic correlation between adaptation to the novel environment (locomotor activity at 30 min subtracted from locomotor activity at 5 min) and β-ED levels in the septum was observed. Estimates of hypothalamic mRNA for the β-ED precursor POMC revealed no effect of strain. Finally, locomotor activity was tested following doses of the μ-opioid antagonist naltrexone. Out of six strains tested (naive to the apparatus), naltrexone dose dependently attenuated locomotor activity in only the strain that showed the highest control level of activity. The results suggest that β-ED levels influence novelty-induced locomotor activity, but that other important genotype-dependent factors are involved. © 1998 Elsevier Science Inc.

Open-field activity Amygdala Hypothalamus Septum Midbrain Pituitary β-Endorphin Genetics Recombinant Inbred mice Naltrexone Proopiomelanocortin

β-ENDORPHIN (β-ED), one of several endogenous opioid peptides, is thought to play a role in open-field activity. Pharmacological manipulation of μ- and/or δ-opioid receptors for which β-ED has high affinity has been demonstrated to alter locomotor behavior. For example, following intracerebroventricular (ICV) β-ED, rats were activated at lower doses but inhibited at higher doses (42). Dermorphin, an opioid peptide from frog skin, and various enkephalin (Enk) analogues administered ICV to mice also were activating but β-ED was inhibitory (34). Intra-VTA microinjections of DAGO, a μ agonist, DPEN, a δ agonist, or DALA, a mixed μ/δ agonist, all stimulated an increase in locomotor activity (23,28). Similarly, intra-Acb DALA or DAGO caused locomotor activation (24).

There appears to be an important influence of stress in the behavioral response to opioid drugs. Selective δ agonists or DAGO given intra-VTA were activating in a familiar environment, but, in a novel environment, DAGO was found to be inhibitory (7). Intra-VTA DAGO also was found to inhibit exploratory behavior in a novel environment, as evidenced by a reduction in the number of open-arm entries in the elevated plus maze (7). Studies with naltrexone or naloxone, selective μ antagonists, generally are in support of an activating role for endogenous opioids, but the novelty of the situation appears to be important (39).

Environmental stress has been demonstrated to elicit a decrease in β-ED levels, generally regarded as release of the

Requests for reprints should be addressed to Richard A. Radcliffe, Ph.D., Institute for Behavioral Genetics, University of Colorado, Campus Box 447, Boulder, CO 80303-0447.

peptide followed by extracellular degradation. For example, a decrease in β-ED from the diencephalon of rats (hypothalamus, thalamus, and preoptic area) was detected following a series of stressors ranging from mild (novel environment) to intense [inescapable foot shock; (22)]. It was noted that the decrease only occurred when the stressor was novel (22). Thus, exogenous and endogenous β-ED–mediated behaviors appear to be influenced by the presence of stress.

Differences in densities of opioid receptors were found in mice selectively bred for stress-induced analgesia (31) and, although not directly tested, a genetically mediated difference in receptors was postulated as causing divergent behavioral responses to morphine (4,5,29). Several quantitative trait loci (QTL) were detected in an analysis of behavioral responses to morphine (morphine preference, analgesia, hypothermia, hypoactivity, and Straub tail), suggesting that these behaviors were influenced by multiple genes (3). A QTL for morphine-induced analgesia mapped at 5 cM on chromosome 10 near where the gene for the μ-opioid receptor (*Oprm*) is located and another, for morphine consumption, was located on chromosome 12 at 3–10 cM where the *Pomc1* locus is found (3, 4). QTL mapping of locomotor behavior identified a QTL near *Pomc1* and, in fact, a *Pomc1* polymorphism was discovered (35,36). Variant *Pomc1* alleles could influence POMC peptide levels and, indeed, genetically mediated differences have been observed. Pit ACTH and β-ED were found to differ among five inbred strains of mice (9) and hypothalamic β-ED was in great excess in genetically obese mice compared to their lean counterparts (27).

Experiments presented in this report were designed to test the hypothesis that central β-ED mediates genetic differences in novelty-induced locomotor behavior. This was accomplished with the use of the LSXSS recombinant inbred (RI) strains. RI strains are created from 20 generations of brother–sister matings of an F_2 derived from two inbred strains (40). This effectively creates a panel of inbred strains, each of which contains a unique combination of the progenitor strain alleles. Long-Sleep (LS) and Short-Sleep (SS) lines of mice, selected for their differential sensitivity to ethanol (30), were used to create one such RI panel (11). Although the LS and SS show similar levels of open-field activity (13), the LSXSS RI strains show a broad, continuous distribution for this behavior and a significant portion of the strain variation can be accounted for by genetic factors (35). Thus, 6 out of the 25 RI strains were selected to study the relationship between β-ED and open-field activity. These six strains were selected based on their previuosly described divergent locomotor behavior (35) and on their availability (many of the strains are poor breeders and available only in limited quantities). Levels of β-ED in the hypothalamus (Hyp), amygdala (Amg), midbrain (MB), medial septum (Sep), and pituitary (Pit) were measured by radioimmunoassay in groups of subjects that were either untreated or had been exposed to a novel environment. Genetic correlations between locomotor activity and peptide levels were determined. In addition, mRNA levels for the β-ED precursor POMC were estimated to determine if differences in peptide levels were mediated at the transcriptional level. Finally, doses of naltrexone were administered to separate groups of mice to examine the effect of μ-receptor antagonism on novelty-induced locomotor behavior.

METHOD

Animals

Male LSXSS RI mice (strains 9, 18, 19, 23, 22, and 32) were obtained from the Institute for Behavioral Genetics (IBG; Boulder, CO) and were between 60 and 80 days of age at the time of testing. The animals were transported from IBG to a holding facility in the School of Pharmacy at the University of Colorado Health Sciences Center (Denver, CO; approx. 25 miles) where they were maintained in a 12 L:12 D cycle in an environment of constant temperature and humidity (22°C, 40% humidity) for at least 4 days before being tested.

Open-Field Behavior

Testing took place between 0800 and 1200 h. Subjects, completely naive to the test procedure, were placed into an automated open-field testing apparatus (Omnitech, Columbus, OH) consisting of a clear Plexiglas square box (40 × 40 × 30 cm) crisscrossed by eight photobeam cells on each side. This box was completely enclosed within an unlighted wooden box (64 × 64 × 56 cm) fitted with a fan that provided 70 dB of background noise. Beam interruptions were stored to an electronic file. Activity was recorded in 5-min blocks for a total of 30 min. Preliminary studies indicated that activity recorded in not more than 5-min time blocks provided an equally accurate measure of the rate of change in locomotion as time periods of less than 5 min (not shown). Following the 30-min test, subjects were returned to their home cage. The Plexiglas box was wiped down with a dilute solution of Roccal (an antiseptic cleaning agent) between subjects. Total distance, measured in the apparatus as the distance traveled within a horizontal plane, was used to represent locomotor activity. For the μ antagonist study, subjects were given naltrexone-HCl IP (0.015 ml/g body weight; prepared fresh on a daily basis in normal saline) and immediately placed into the activity monitor. A separate group of naive animals was used for determinations of β-ED and POMC mRNA. These animals were removed from the home cage and placed into the activity monitor. Immediately following behavioral testing, subjects were transported to a separate room for brain dissections.

β-ED Radioimmunoassay

Subjects were placed into a transport cage either directly from their home cage (naive) or immediately following 30 min of activity testing (novel environment) and taken to a separate room for dissections. The animals were sacrificed by cervical dislocation and the brain was quickly removed into ice-cold saline. After chilling for approx. 1 min, the brain was placed onto a chilled aluminum block and the Pit, Amg, Sep, MB, and one-half of the Hyp were dissected. Brain regions were identified according to the atlas of Slotnick and Leonard (41). The regions were placed into tared Eppendorf tubes containing 10–20 vol of 0.1 N HCl and weighed. Tubes were placed into a boiling water bath for 5 min and then stored at −20°C. After 1–3 months, the samples were thawed, homogenized, and centrifuged at 20,000 × *g* for 20 min. The resulting supernatant was transferred into new tubes and lyophilized. The lyophilate was reconstituted in RIA buffer (Na_2HPO_4, 45 mM; NaH_2PO_4, 55 mM; NaCl, 150 mM; NaN_3, 1 g/l; bovine serum albumin, 5 g/l; Traysylol, 5 U/l) in preparation to be assayed.

β-ED immunoreactivity (β-ED-ir) was determined by a standard double-antibody radioimmunoassay (RIA). According to the manufacturer, the β-ED antiserum (Sigma, St. Louis, MO) was only slightly crossreactive with the following peptides: α-endorphin (15%), γ-endorphin (10%), [Leu^5]-enkephalin (0.01%), and [Met_5]-enkephalin (0.01%). Standards (15 to 1000 pg) and samples, both prepared in RIA buffer, in-

cubated with β-ED antiserum (1:100 dilution as per manufacturer's instructions) overnight at 4°C. 3-[^{125}I]iodotyrosol-β-ED (~30,000 dpm; 2000 Ci/mmol; Amersham) was added followed by another overnight incubation at 4°C. After a 20-min incubation with IgGSorb (killed *S. aureus* cells containing an abundance of protein A on their cell surface; the Enzyme Center, Malden, MA), 1% Tween 20 in normal saline was added and the tubes were centrifuged at 3000 × *g* for 45 min. The supernatant was removed and disposed and radioactivity in the pellet was determined on a model 4/200 gamma counter (Micromedic Systems). Sample d.p.m. were compared to standards following a log/logit transformation. The linear range of the transformed standards (i.e., between the EC_{20} and EC_{80}) was from 50 to 800 pg β-ED with an IC_{50} of 160 pg. Samples were assayed in duplicate with two dilutions, and only values that fit into the linear portion of the standard curve were used for analyses. The data are expressed as pg/mg wet tissue weight except for the Pit, for which accurate weights were not attainable. β-ED values in the Pit, therefore, are expressed as pg/Pit.

POMC RNase Protection Assay

In the same mice that were used for β-ED levels, the Pit from every other mouse and the other half of the Hyp were removed for determination of POMC mRNA. Total RNA was extracted by the guanidine thiocyanate method. Following dissection, tissue was immediately placed into denaturing solution (guanidine thiocyanate, 4 M; sodium citrate, 42 mM; N-lauryl sarcosine, 0.83%; 2-mercaptoethanol, 0.2 mM). The tissue was homogenized and the resulting solution was brought to pH 4.0 with sodium acetate (2 M). Phenol:chloroform:isoamyl alcohol (25:24:1) was added and the tubes were thoroughly mixed and incubated on ice for 15 min. The samples were centrifuged (10,000 × *g* for 15 min) and the aqueous supernatant was removed to a new tube. One volume of isopropanol was added and the samples were incubated at −20°C for at least 1 h. The samples were centrifuged (16,000 × *g* for 15 min) and the resulting pellet was resuspended in denaturing solution. Isopropanol again was added and the samples incubated at −20°C for at least 1 h. Following centrifugation (16,000 × *g* for 15 min), the pellet was washed with 75% ethanol and resuspended in DEPC-H_2O. The amount of total RNA in the sample was quantified by measuring UV absorbance at 260 nm.

The plasmid rEX3* contained a 1.3 kb fragment of POMC cDNA that had been inserted into the polyclonal Bluescript Sk ± vector by Dr. M. J. Low (Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR) who generously provided a small amount for these experiments. It was inserted such that linearization at a unique POMC *XhoI* cleavage site and subsequent transcription by T7 RNA polymerase gave a 200 bp fragment that was complimentary to 170 bp of POMC cDNA. Competent JM109 cells (Promega, Madison, WI) were transformed by the heat-shock method as per the manufacturer's instruction. Cells were grown overnight at 37°C on agar plates (Lauria-Burtoni medium, LB) containing ampicillin (100 μg/ml). Six isolated colonies were selected for overnight growth in liquid LB medium also containing ampicillin (100 μg/ml). Plasmid DNA was isolated from the cells by a standard phenol-chloroform extraction procedure. It was verified in all six colonies that cleavage with either *XhoI* or *PvuII* (both are unique cleavage sites in rEX3*) gave a single product of approximately 4500 bp and cleavage with both enzymes yielded two products of the appropriate sizes (data not shown). This was determined by electrophoresis of the reaction products, alongside DNA size markers, on a 1% agarose gel containing ethidium bromide followed by inspection under UV light. An aliquot of the plasmid DNA was linearized with *XhoI* and stored at −20°C until use.

^{32}P-labeled riboprobes for POMC and mouse β-actin (from the Ambion RPAII kit, Austin, TX) were prepared with the use of the MAXIscript kit (Ambion, Austin, TX). T7 RNA polymerase (5 U) was added to 1 μg of linearized plasmid in the presence of a limiting concentration of [α-^{32}P]CTP (3 μM). ATP, UTP, and GTP were each at a concentration of 500 μM and the reaction mix contained RNase inhibitor (5 U). The reaction proceeded for 45 min at 37°C and was followed by incubation for another 10 min in the presence of DNase I (2 U) to remove template material. The labeled probes were gel purified by electrophoresis on a 5% polyacrylamide denaturing gel followed by excision of the most intense bands corresponding to the correct size and overnight incubation (room temp.) in elution buffer (RPAII kit). The incorporation of [α-^{32}P]CTP was typically 30–40% and the specific activities of the probes were calculated to be approx. 5×10^8 c.p.m./μg RNA.

The RPA II kit (Ambion, Austin, TX) was used for ribonuclease solution/hybridization protection assays. Sample RNA (approx. 5 μg of total RNA) and both riboprobes (2 fmol) were coprecipitated with ethanol. The pellet was resuspended with hybridization solution and incubated overnight at 45°C to hybridize the probes to their respective complimentary sequence. Digestion of all single-stranded RNA was accomplished with the addition of RNase mixture and incubation at 37°C for 30 min. The samples were centrifuged and the pellet resuspended in gel loading buffer. Aliquots of the samples were electrophoresed on a 5% denaturing polyacrylamide gels. Dried gels were imaged and bands corresponding to the protected fragments of POMC and β-actin were quantified using a Phosphoroimager SI image analysis system (Molecular Dynamics, Sunnyvale, CA).

Data Analysis

Two-way ANOVA was used to determine between-subjects effects for strain or treatment and within-subjects effects for time for novelty- or naltrexone-induced locomotor activity, respectively, and to determine between-subjects effects for strain and treatment for naive and novelty-induced levels of β-ED. One-way ANOVA was used for the analysis of between-strain effects for levels of POMC mRNA. Pearson product-moment correlations between RI strain means were used to calculate genetic correlation coefficients. Results of statistical analyses are shown in the figure legends. Analyses were performed using SPSS for Windows (v. 6.1).

RESULTS

Six LSXSS RI strains representing extremes from both ends of the locomotor activity distribution were selected to examine the role of β-ED in spontaneous locomotor activity. Their locomotor activity time courses are shown in Fig. 1. Two-way ANOVA indicated that there were significant effects of strain, time, and a significant strain by time interaction. There were significant simple main effects of time for all strains except 18. The data for all of the strains were similar to the original data from which these strains were chosen (35) except strain 19, which showed considerably lower activity in this experiment. The interaction effect is easily discerned by examination of the differential activity time course of the

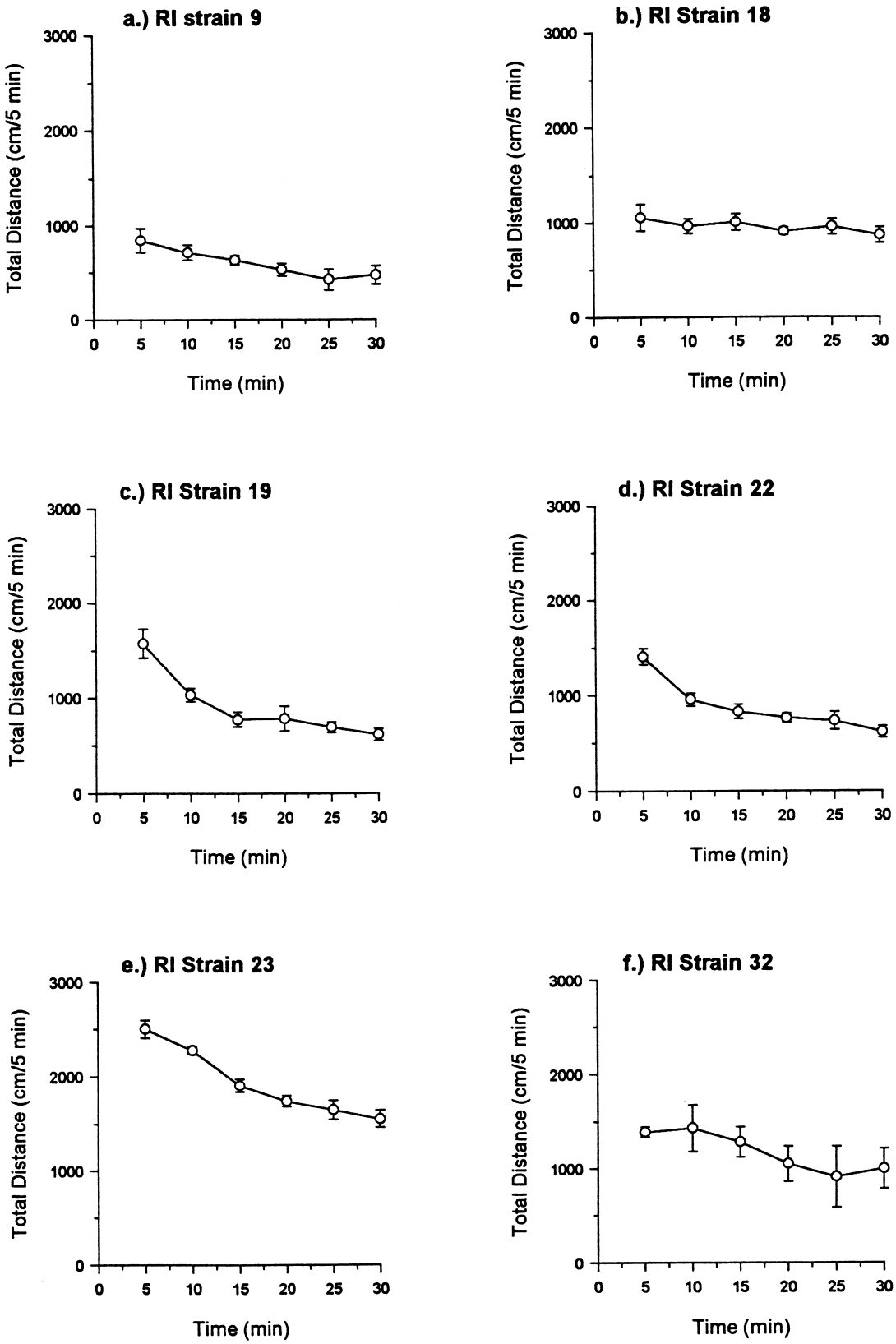


FIG. 1. Open-field activity in LSXSS RI strains. Subjects were naive to the apparatus and received no other treatment prior to testing. Shown are the means (±SEM, $n = 4$–6) of distance traveled (cm) in 5-min blocks. Two-way ANOVA indicated significant effects of strain, $F(31, 5) = 46.2$, $p < 0.0001$, of time, $F(160, 5) = 47.2$, $p < 0.0001$, and a significant strain by time interaction, $F(25, 130) = 5.0$, $p < 0.0001$.

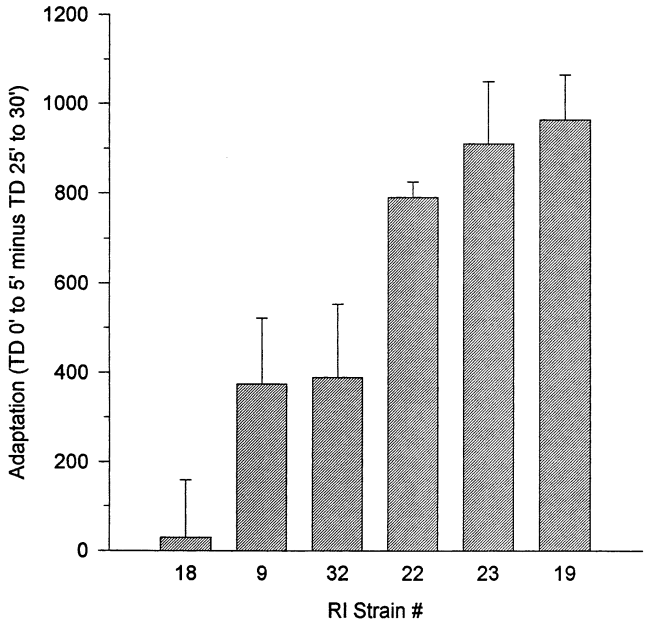


FIG. 2. Locomotor adaptation in LSXSS RI strains. Adaptation was calculated as the total distance traveled (cm) during the time period 25 to 30 min subtracted from total distance during the 0- to 5-min period. Shown are the difference scores (mean ± SEM, $n = 4$–5) derived from results shown in Fig. 1. One-way ANOVA indicated a significant strain effect, $F(35, 5) = 8.88$, $p < 0.0001$.

strains. For example, strain 18 shows virtually no change in activity over the 30-min time course whereas strain 19 decreased by over 60%. The significant interaction between time and strain prompted the examination of each individual time period as an independent measure. In addition, it was desirable to investigate β-ED levels with relation to these differences. Thus, a variable representing this phenomenon was introduced and will be referred to as locomotor adaptation. Adaptation was quantified as the difference score between distance traveled in the first 5 min and the last 5 min of the 30-min test. Adaptation was found to have a significant RI strain effect (Fig. 2).

β-ED-ir was determined by RIA in various brain regions and in the Pit either before or after a 30-min exposure to a novel environment (Fig. 3). There was a greater than 16-, five-, and threefold difference between the highest and lowest strains in the Sep, Amg, and MB, respectively, while a less than twofold difference was seen in the Hyp and Pit. Two-way ANOVA indicated significant strain effects for all brain regions and a significant strain by treatment interaction for Amg and MB. There was no overall treatment effect for any region, although a simple main effects analysis revealed that in some strains there was either a significant increase or decrease in β-ED-ir following exposure to a novel environment. This is shown with asterisks in Fig. 3. Strain 9 shows especially large decreases in Sep and Amg (approximately sevenfold and fivefold, respectively). There was no effect of strain or treatment on β-ED-ir in the Pit. In addition, comparison of β-ED-ir among brain regions from naive animals revealed that there was a general trend of high, positive correlations between most areas, but none of these correlations reached statistical significance (not shown).

Quantities of POMC mRNA were estimated using a solution-hybridization ribonuclease protection assay. The main tissues where POMC mRNA is expressed are the Hyp and the Pit. In this study, there was a significant effect of strain on β-ED-ir only in the Hyp and not the Pit. Thus, estimates of mRNA levels were determined only in the Hyp (Fig. 4). POMC mRNA was normalized to the amount of β-actin mRNA whose tissue level was assumed to remain constant across brain regions and across RI strains. No significant effect of RI strain on the POMC/β-actin mRNA ratio was found. In addition, hypothalamic β-ED-ir and POMC mRNA do not correlate particularly well. There is, however, an indication that POMC mRNA predicts levels of β-ED-ir. The two strains with the highest β-ED-ir in the Hyp also have the highest levels of POMC mRNA (strains 9 and 32), whereas those strains with the lowest hypothalamic β-ED-ir also have low levels of mRNA (strains 19 and 23). In addition, there were high, positive genetic correlations, albeit nonsignificant, with β-ED-ir in the Sep ($r = 0.65$, $p = 0.16$) and Amg ($r = 0.68$, $p = 0.13$).

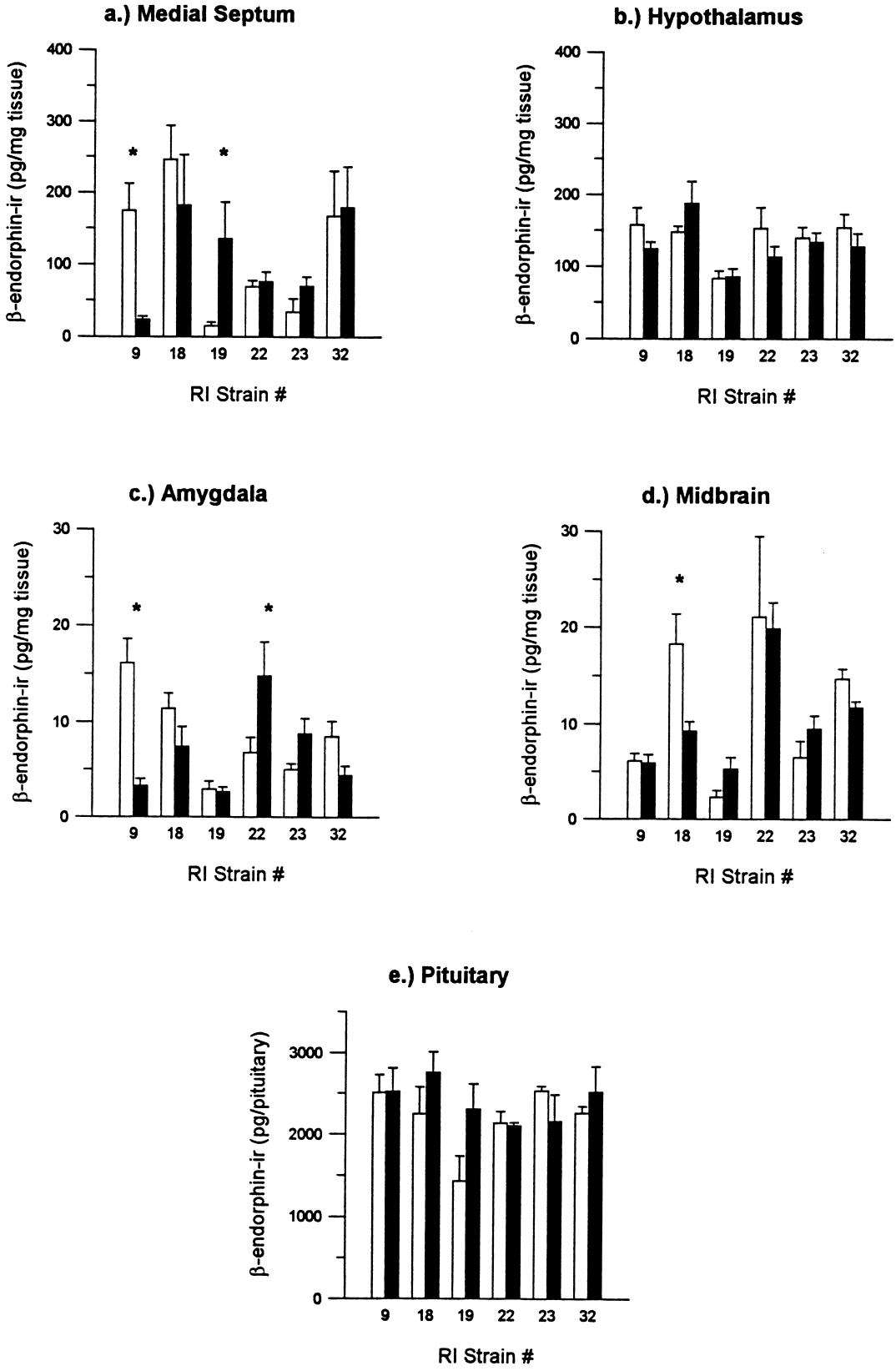
Data from the above studies were examined for the presence of genetic correlations between β-ED-ir and locomotor activity. Locomotor behavior was broken down into separate time blocks and the adaptation measure was also considered. Values of p were corrected for multiple testing by the method of Bonferroni and, thus, the only significant correlation observed was between locomotor adaptation and naive β-ED-ir in the Sep. This is shown as a scatterplot in Fig. 5. This correlation was negative, indicating that lower β-ED-ir at the start of the test was predictive of a greater decline in locomotor activity after 30 min. It should be noted that, although not statistically significant, an additional high, negative correlation was observed between naive β-ED levels in the Amg and locomotor adaptation ($r = -0.82$, $p > 0.05$).

The results of β-ED-ir determinations suggested a modest genetic relationship between β-ED levels and variation in locomotor activity. If this was the case, then strains divergent in locomotor activity should be differentially responsive to drugs which act on the β-ED system. Thus, doses of naltrexone, a moderately selective μ antagonist, were administered to a separate group of the selected RI strains. The results of these experiments are shown in Fig. 6. A time by treatment two-way ANOVA for each strain indicated a significant time effect for all of the strains, but only a significant treatment effect for RI 23 (Fig. 6e). There was clearly a dose-dependent effect of naltrexone independent of time, but it was only observed in strain 23.

DISCUSSION

Experiments in this report were designed to test the hypothesis that genetically mediated differences in brain β-ED-ir contribute to genetic variation in locomotor activity. β-ED–related measures were determined in behaviorally divergent LSXSS RI strains. It was found that there was a strain effect for levels of β-ED-ir in the Hyp and β-ED terminal areas but not in the Pit. Exposure to a novel environment had no overall effect on β-ED-ir, although both increases and decreases were observed in some isolated cases. There was no effect of strain on estimates of POMC mRNA levels in the Hyp. β-ED-ir from the Sep of naive animals was highly correlated to locomotor adaptation. Finally, naltrexone attenuated novelty-induced locomotor activity in only one out of the six strains tested.

Levels of β-ED-ir were found to be strain dependent in all brain regions, but not in the Pit. This result supports the hypothesis that central β-ED levels are under genetic regulation particularly in the Amg and Sep where the greatest between-


a.) Medial Septum
b.) Hypothalamus
c.) Amygdala
d.) Midbrain
e.) Pituitary
β-endorphin-ir (pg/mg tissue)
β-endorphin-ir (pg/pituitary)
RI Strain #
9
18
19
22
23
32
*

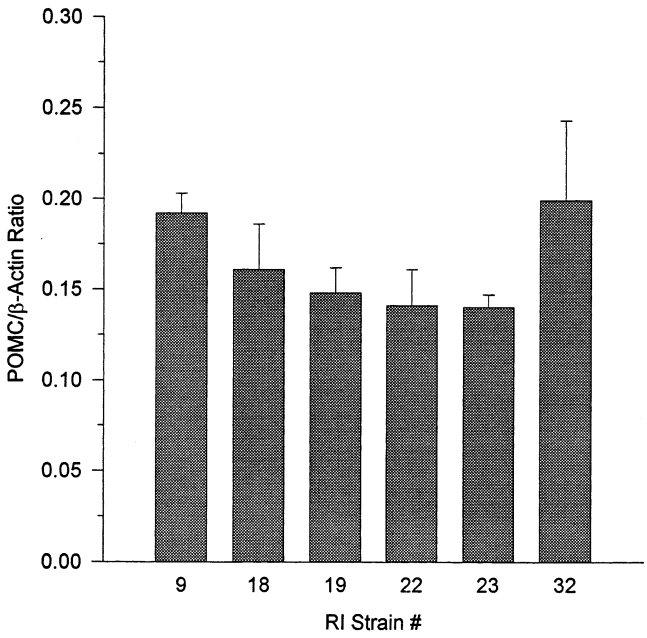


FIG. 4. Estimated POMC mRNA levels from the hypothalamus of LSXSS RI strains. mRNA amounts were determined by a solution-hybridization RNase protection assay. POMC mRNA levels were normalized to β-actin mRNA as described under the Method section. Values shown represent the mean ± SEM(n = 4–6) of POMC mRNA/β-actin mRNA. There was no effect of strain.

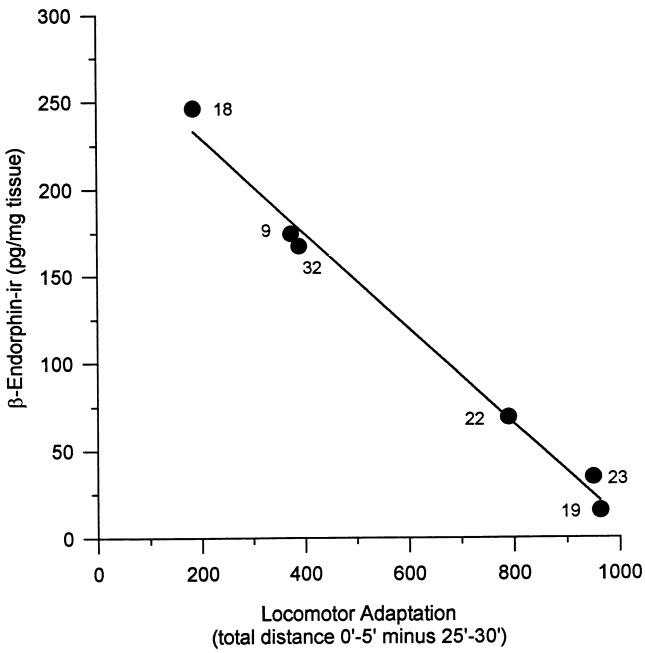


FIG. 5. Scatterplot of genetic correlation between locomotor adaptation and β-ED-ir in the medial septum among LSXSS RI strains. RI strain means (n = 4–6) were used to determine Pearson product-moment correlation coefficients ($r = -0.99$, $p < 0.002$). RI strain number is shown beside each data point. Solid line indicates least-squares linear regression.

strain variation was observed. Variation in neuropeptide levels may be accounted for by differences in transcription, translation, posttranslational processing, release (or secretion), or degradation. Recently, as a follow-up to a QTL analysis of locomotor activity in the LSXSS RI strains (35), a *Pomc1* polymorphism was described (36). However, it was found that this variant allele apparently did not contribute to differences in locomotor behavior. A comparison of the current results with the strain distribution pattern of the two *Pomc1* alleles indicates that the polymorphism also does not appear to contribute to genetically mediated levels of β-ED (36). Because only a small portion of the *Pomc1* promoter was analyzed, one cannot exclude the possibility that another polymorphism in a different regulatory region of *Pomc1*, possibly as much as or more than 10,000 bases upstream of the *Pomc1* coding region (38), contributes to variation in β-ED levels. However, it is likely that other *Pomc1* polymorphisms would be linked to those that were found, and so it is more probable that strain-dependent differences in β-ED levels are due to polymorphic genes affecting other proteins, for example, transcriptional factors or POMC processing enzymes. In any case, the lack of significant genetic correlations among brain regions and the Pit suggests that the tissue-specific nature of β-ED expression is genotype dependent.

It has been reported that novelty causes a decrease in brain β-ED, interpreted as stimulated release and subsequent peptide metabolism (21,22). The current findings, while not in complete disagreement, indicate that other factors may be involved. A large decrease was noted following exposure to a novel environment in the Sep, Amg, and MB, but only in one strain for each region. Most of the strains showed no change and one strain showed an increase in β-ED-ir in the Sep and Amg. The time course of these changes, as short as 3 min in one report (22), suggest that transcription and translation are probably not major factors. If decreased levels are an indicator of peptide release, then the present results suggest that there is an important genotype-dependence on such functions as posttranslational processing, extracellular metabolism, or the release process itself.

It is perhaps notable that there was a relationship, albeit weak, between POMC mRNA and β-ED-ir in the Amg and Sep (correlation coefficients were very low for other regions). This observation suggests that variation in β-ED-ir can be attributed, at least in part, to differential expression (or stability) of POMC mRNA. Furthermore, the finding that β-ED-ir was correlated between these two regions suggests they are under pleiotropic genetic regulation. It is possible that some genetically mediated aspect of POMC mRNA stability or

←

FIG. 3. β-ED-ir from brain regions of LSXSS RI strains. Values shown represent the mean (±SEM, n = 4–6) β-ED-ir/wet tissue weight for each brain region and β-ED-ir/Pit for the Pit. β-ED-ir was determined by RIA before (open bars) and after (solid bars) a 30-min exposure to a novel environment. The automated open-field activity monitor was used as a novel environment. A strain by treatment two-way ANOVA indicated: significant effects of strain in the Hyp, $F(61, 5) = 3.56$, $p < 0.01$, MB, $F(57, 5) = 12.59$, $p < 0.0001$, Amg, $F(59, 5) = 4.91$, $p < 0.001$, and Sep, $F(41, 5) = 4.64$, $p < 0.005$, and a significant strain by treatment interaction in the MB, $F(5, 46) = 2.48$, $p < 0.05$, Amg, $F(5, 48) = 7.92$, $p < 0.0001$, and Sep, $F(5, 30) = 2.60$, $p < 0.05$. No overall effect of treatment was found for any region but significant simple main effects ($p < 0.01$) of treatment (i.e., novel environment) within a strain were detected (indicated by asterisks).

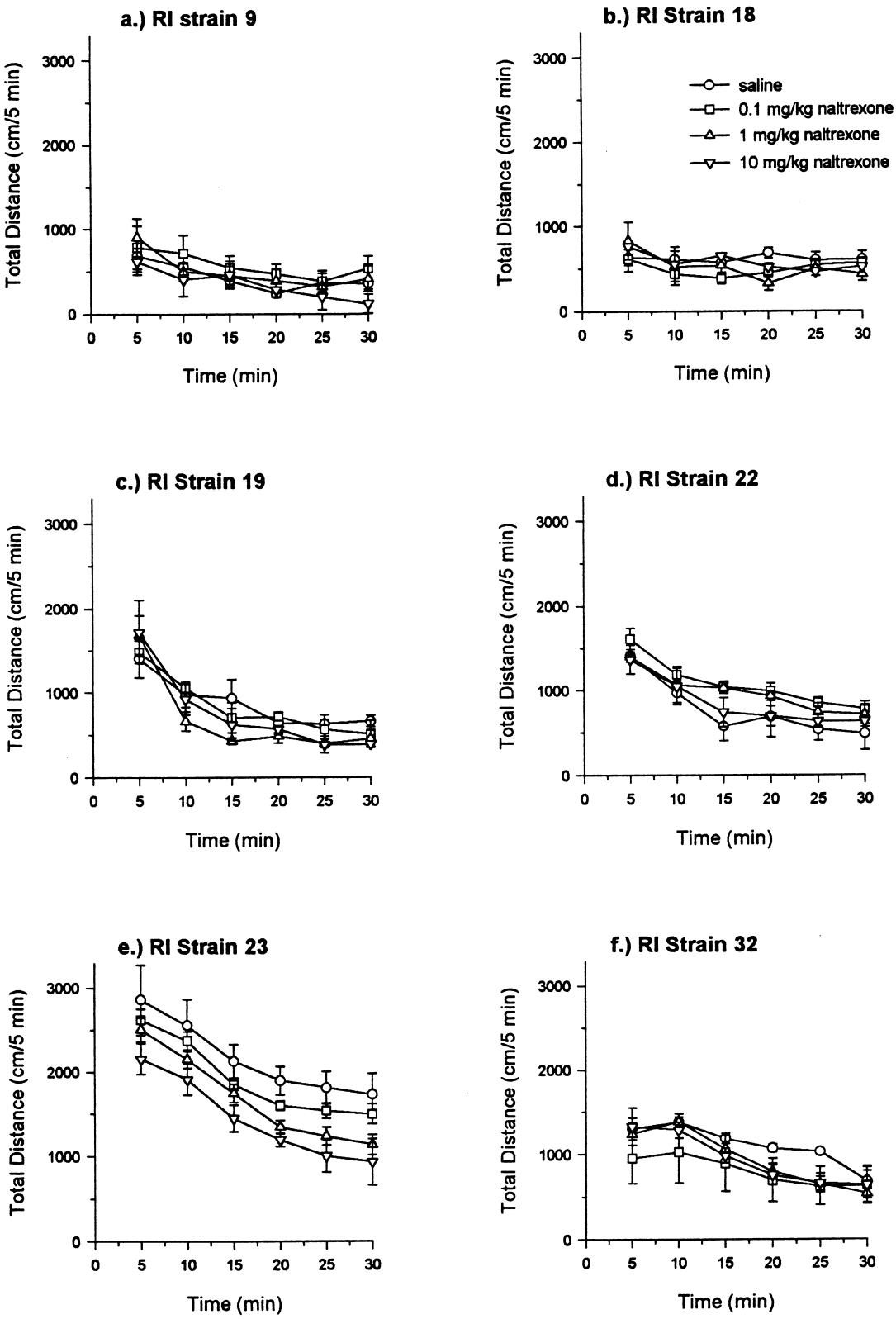


FIG. 6. Locomotor response to doses of naltrexone in LSXSS RI strains. Shown are the means (±SEM, $n = 5$–6) of distance traveled (cm) in 5-min blocks. Subjects were naive to the test procedure and received naltrexone (IP) immedietely preceeding activity testing in doses shown in b. One-way ANOVA indicated no significant effect of naltrexone except in strain 23, $F(19, 3) = 6.73$, $p < 0.004$.

transcription is influential only in cells projecting to these regions. However, the lack of a strain effect on estimates of mRNA precludes one from making any definitive statements. The absence of an mRNA strain effect may have been because of the heterogeneous nature of the hypothalamic tissue from which mRNA was estimated (20); cell-specific effects on mRNA content may have been obscured. In support of this possibility, POMC mRNA levels were found to be differentially responsive to restraint stress among distinct groups of arcuate neurons (2). Terminal projection areas of specific cells or cell groups, however, have not been anatomically delineated.

The observed genetic correlation between locomotor adaptation and β-ED-ir in the Sep and the nonsignificant but high correlation between Amg β-ED-ir and adaptation support the hypothesis that β-ED influences locomotor activity in a genotype-dependent fashion. It is possible that animals with higher levels of β-ED are less anxious and thus maintain a constant level of exploratory activity throughout the 30-min test. The Sep is thought to reduce novelty-induced anxiety, as demonstrated by enhanced exploratory behavior in the elevated plus-maze following lesioning of the Sep (33). Moreover, although fear and anxiety have not been examined in the Sep specifically with relation to opioid activity, intra-Sep β-ED or morphine attenuated learning capacity, but only in a novel environment (6,19,37), similarly as in the Amg (16), an important mediator of learning under the context of emotional arousal (10,15). β-ED likely plays a role in this function of the Amg, as suggested by the observations that intra-Amg infusions of opiate agonists reduced physiological measures of fear and also had anxiolytic effects (14,17,18). The current results suggest that β-ED activity in the Sep and Amg, at least as a function of peptide content, may be working in a correlated fashion to reduce levels of fear or anxiety, thereby enhancing exploratory behavior resulting in less of a decrease in locomotion over time.

The results of experiments with naltrexone suggest that naltrexone-mediated alterations in locomotor behavior are genotype dependent. However, only one out of the six strains were affected by naltrexone, which does not support the hypothesis that differences in activity are mediated exclusively through strain-related differences in β-ED. This finding could be explained by several possibilities. First, other endogenous opioids, for example, Enk, may be involved in the behavioral response to novelty (25,26,43,44). Enk is only slightly more selective for δ than for μ receptors, and other pro-Enk products have high affinity for μ (1) and, thus, naltrexone would not distinguish between β-ED- vs. Enk-associated receptors. Also, naltrexone, particularly the two higher doses, may have been blocking other opioid receptor subtypes. An interaction between the antagonism of both Enk and β-ED activity at μ and δ receptors, complicated by a potential genetic effect on both Enk and β-ED systems (8,31,32), may have contributed to the current results. Second, strain 19 was selected for use in this study because it had exceptionally high activity. For some unknown reason, that strain's activity was much lower in this experiment effectively reducing the number of high-activity strains to one—strain 23—whose activity was reduced by naltrexone. Third, μ-receptor densities have been found to be genotype dependent in other mouse strains (31). Variation in levels of this receptor among the LSXSS RI strains may have contributed to the results of this study. Finally, genetic mechanisms that influence β-ED levels may also influence the tissue content of other POMC peptides in a correlated fashion. Thus, it is possible that variation in activity, which is postulated to be due in part to β-ED levels, may be influenced by other POMC peptides (12).

ACKNOWLEDGEMENTS

This work was supported by USPHS Grant AA03527 and in part by the Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, in partial fulfillment of the requirements for a Ph.D. degree.

REFERENCES

1. Akil, H.; Bronstein, D.; Mansour, A.: Overview of the endogenous opioid systems: Anatomical, biochemical and functional issues. In: Rodgers, R. J.; Cooper, S. J., eds. Endorphins, opiates and behavioural processes. New York: John Wiley & Sons Ltd.; 1988:1–23.
2. Baubet, V.; Fevre-Montange, M.; Gay, N.; Debilly, G.; Bobillier, P.; Cespuglio, R.: Effects of an acute immobilization stress upon proopiomelanocortin (POMC) mRNA levels in the mediobasal hypothalamus: A quantitative in situ hybridization study. Brain Res. Mol. Brain Res. 26:163–168; 1994.
3. Belknap, J. K.; Crabbe, J. C.: Chromosome mapping of gene loci affecting morphine and amphetamine responses in BXD recombinant inbred mice. Ann. NY Acad. Sci. 654:311–323; 1992.
4. Belknap, J. K.; Mogil, J. S.; Helms, M. L.; Richards, S. P.; O'Toole, L. A.; Bergeson, S. E.; Buck, K. J.: Localization to chromosome 10 of a locus influencing morphine analgesia in crosses derived from C57BL/6 and DBA/2 strains. Life Sci. 57:PL117–PL124; 1995.
5. Belknap, J. K.; Noordewier, B.; Lame, M.: Genetic dissociation of multiple morphine effects among C57BL/6J, DBA/2J and C^{3}H/HeJ inbred mouse strains. Physiol. Behav. 46:69–74; 1989.
6. Bostock, E.; Gallagher, M.; King, R. A.: Effects of opioid microinjections into the medial septal area on spatial memory in rats. Behav. Neurosci. 102:643–652; 1988.
7. Calenco-Choukroun, G.; Dauge, V.; Gacel, G.; Feger, J.; Roques, B. P.: Opioid delta agonists and endogenous enkephalins induce different emotional reactivity than mu agonists after injection in the rat ventral tegmental area. Psychopharmacology (Berlin) 103:493–502; 1991.
8. Castellano, C.: Strain dependent effects of the enkephalin analogue FK 33–824 on locomotor activity in mice. Pharmacol. Biochem. Behav. 15:729–734; 1981.
9. Crabbe, J. C.; Allen, R. G.; Gaudette, N. D.; Young, E. R.; Kosobud, A.; Stack, J.: Strain differences in pituitary β-endorphin and ACTH content in inbred mice. Brain Res. 219:219–223; 1981.
10. Davis, M.: The role of the amygdala in fear and anxiety. Annu. Rev. Neurosci. 15:353–375; 1992.
11. DeFries, J. C.; Wilson, J. R.; Erwin, V. G.; Petersen, D. R.: LSXSS recombinant inbred strains of mice: Initial characterization. Alcohol. Clin. Exp. Res. 13:196–200; 1989.
12. de Wied, D.; Jolles, J.: Neuropeptides derived from pro-opiocortin: Behavioral, physiological, and neurochemical effects. Physiol. Rev. 62:976–1059; 1982.
13. Erwin, V. G.; Radcliffe, R.; Jones, B. C.: Chronic ethanol consumption produces genotype-dependent tolerance to ethanol in LS/lbg and SS/lbg mice. Pharmacol. Biochem. Behav. 41:275–281; 1992.
14. File, S. E.; Rodgers, R. J.: Partial anxiolytic action of morphine sulphate following microinjection into the central nucleus of the amygdala in rats. Pharmacol. Biochem. Behav. 11:313–318; 1979.
15. Gallagher, M.; Chiba, A. A.: The amygdala and emotion. Curr. Opin. Neurobiol. 6:221–227; 1996.
16. Gallagher, M.; Kapp, B. S.: Manipulation of opiate activity in the amygdala alters memory processes. Life Sci. 23:1973–1977; 1978.

17. Gallagher, M.; Kapp, B. S.; Pascoe, J. P.: Enkephalin analogue effects in the amygdala central nucleus on conditioned heart rate. Pharmacol. Biochem. Behav. 17:217–222; 1982.
18. Gallagher, M.; Kapp, B. S.; McNall, C. L.; Pascoe, J. P.: Opiate effects in the amygdala central nucleus on heart rate conditioning in rabbits. Pharmacol. Biochem. Behav. 14:497–505; 1981.
19. Gallagher, M.; Meagher, M. W.; Bostock, E.: Effects of opiate manipulations on latent inhibition in rabbits: Sensitivity of the medial septal region to intracranial treatments. Behav. Neurosci. 101:315–324; 1987.
20. Ibata, Y.; Kawakami, F.; Okamura, H.; Obata-Tsuto, H. L.; Morimoto, N.; Zimmerman, E. A.: Light and electron microscopic immunocytochemistry of β-endorphin/β-LPH-like immunoreactive neurons in the arcuate nucleus and surrounding areas of the rat hypothalamus. Brain Res. 341:233–242; 1985.
21. Izquierdo, I.; Souza, D. O.; Carrasco, M. A.; Dias, R. D.; Perry, M. L.; Eisinger, S.; Elisabetsky, E.; Vendite, D. A.: Beta-endorphin causes retrograde amnesia and is released from the rat brain by various forms of training and stimulation. Psychopharmacology (Berlin) 70:173–177; 1980.
22. Izquierdo, I.; Souza, D. O.; Dias, R. D.; Perry, M. L.; Carrasco, M. A.; Volkmer, N.; Netto, C. A.: Effect of various behavioral training and testing procedures on brain beta-endorphin-like immunoreactivity and the possible role of beta-endorphin in behavioral regulation. Psychoneuroendocrinology 9:381–389; 1984.
23. Kalivas, P. W.; Abhold, R.: Enkephalin release into the ventral tegmental area in response to stress: Modulation of mesocorticolimbic dopamine. Brain Res. 414:339–348; 1987.
24. Kalivas, P. W.; Bronson, M.: Mesolimbic dopamine lesions produce an augmented behavioral response to enkephalin. Neuropharmacology 24:931–936; 1985.
25. Katz, R. J.; Carroll, B. J.: Enkephalin-dopamine interactions in behavioral arousal. Prog. Neuropsychopharmacol. 3:395–398; 1979.
26. Katz, R. J.: Opiate stimulation increases exploration in the mouse. Int. J. Neurosci. 9:213–215; 1979.
27. Khawaja, X. Z.; Chattopadhyay, A. K.; Green, I. C.: Increased beta-endorphin and dynorphin concentrations in discrete hypothalamic regions of genetically obese (ob/ob) mice. Brain Res. 555:164–168; 1991.
28. Latimer, L. G.; Duffy, P.; Kalivas, P. W.: Mu opioid receptor involvement in enkephalin activation of dopamine neurons in the ventral tegmental area. J. Pharmacol. Exp. Ther. 241:328–337; 1987.
29. Marek, P.; Mogil, J. S.; Belknap, J. K.; Sadowski, B.; Liebeskind, J. C.: Levorphanol and swim stress-induced analgesia in selectively bred mice: Evidence for genetic commonalities. Brain Res. 608:353–357; 1993.
30. McClearn, G. E.; Kakihana, R.: Selective breeding for ethanol sensitivity in mice. Behav. Gen. 3:409–410; 1973.
31. Mogil, J. S.; Marek, P.; O'Toole, L. A.; Helms, M. L.; Sadowski, B.; Liebeskind, J. C.; Belknap, J. K.: Mu-opiate receptor binding is upregulated in mice selectively bred for high stress-induced analgesia. Brain Res. 653:16–22; 1994.
32. Oliverio, A.; Castellano, C.: Behavioral effects of opiates: A pharmacogenetic analysis. Curr. Dev. Psychopharmacol. 6:45–64; 1981.
33. Pesold, C.; Treit, D.: Excitotoxic lesions of the septum produce anxiolytic effects in the elevated plus-maze and the shock-probe burying tests. Physiol. Behav. 52:37–47; 1992.
34. Puglisi-Allegra, S.; Castellano, C.; Filibeck, U.; Oliverio, A.; Melchiorri, P.: Behavioural data on dermorphins in mice. Eur. J. Pharmacol. 82:223–227; 1982.
35. Radcliffe, R. A.; Jones, B. C.; Erwin, V. G.: Mapping of provisional quantitative trait loci influencing temporal variation in locomotor acvtivity in the LSXSS recombinant inbred strains. Behav. Genet. 28:39–47; 1998.
36. Radcliffe, R. A.; Walter, N. A. R.; Sikela, J. M.; Erwin, V. G.: Discovery of a new *Pomc1* allele in the LSXSS recombinant inbred strains: Relationship to locomotor activity. Behav. Genet. 28:49–55; 1998.
37. Ragozzino, M. E.; Gold, P. E.: Glucose injections into the medial septum reverse the effects of intraseptal morphine infusions on hippocampal acetylcholine output and memory. Neuroscience 68:981–988; 1995.
38. Rubinstein, M.; Mortrud, M.; Liu, B.; Low, M. J.: Rat and mouse proopiomelanocortin gene sequences target tissue-specific expression to the pituitary gland but not to the hypothalamus of transgenic mice. Neuroendocrinology 58:373–380; 1993.
39. Segal, D. S.; Browne, R. G.; Arnsten, A.; Derrington, D. C.; Bloom, F. E.; Davis, A. V.; Guillemin, R.; Ling, N.: Characteristics of β-endorphin-induced behavioral activation and immobilization. In: Usdin, E.; Bunney, W. E.; Kline, N. S., eds. Endorphins in mental health research. New York: Oxford University Press; 1979:307–324.
40. Silver, L. M.: Mouse genetics: Concepts and applications. New York: Oxford University Press; 1995.
41. Slotnick, B. M.; Leonard, C. M.: Stereotaxic Atlas of the albino mouse forebrain. DHEW Publication (ADN) 75–100; 1975.
42. Spanagel, R.; Herz, A.; Bals-Kubik, R.; Shippenberg, T. S.: Beta-endorphin-induced locomotor stimulation and reinforcement are associated with an increase in dopamine release in the nucleus accumbens. Psychopharmacology (Berlin) 104:51–56; 1991.
43. Ukai, M.; Kameyama, T.: The antagonistic effects of naloxone on hypermotility in mice induced by dynorphin-(1–13) using a multidimensional behavioral analysis. Neuropharmacology 23:165–168; 1984.
44. Ukai, M.; Yamada, S.; Kameyama, T.: Naloxone reserves the inhibitory effects of dynorphin A on motor activity in the mouse. Pharmacol. Biochem. Behav. 20:815–818; 1984.