Induction of the Intermediate Pituitary by Stress: Synthesis and Release of a Nonopioid Form of β-Endorphin

Abstract. *β-Endorphin in the intermediate lobe of the pituitary gland is posttranslationally modified to produce opioid inactive peptides. Whether these are metabolites or biologically relevant products has not been known. It was found that repeated stress induces increased biosynthesis and release of β-endorphin–like substances from the intermediate lobe of rats and that opioid-inactive N-acetylated β-endorphin-(1–31) is selectively made and liberated. The possible role of this nonopioid product and the selective release of peptide forms are discussed.*

Posttranslational modifications of neuropeptides can bring about significant changes in their biological activities. However, given that neuropeptides can have multiple active cores, it is difficult to determine a priori whether a given modified form is a "true" end product, subject to release and regulation, or whether it represents an inactive stored metabolite. This question arises in the case of the intermediate lobe of the pituitary, which contains the highest concentration of the common precursor to adrenocorticotropic hormone (ACTH) and β-endorphin-(1–31). The end products of pro-opiomelanocortin in the intermediate lobe are highly modified posttranslationally to produce various forms of acetylated β-endorphin and to convert ACTH to α-melanotropin (α-MSH) and corticotropin-like intermediate lobe protein (*1, 2*). These products are essentially devoid of the opioid (*3*) and steroidogenic activities of β-endorphin-(1–31) and ACTH. Are they inactive metabolites or are they true products, subject to release and specific regulation? The results of this study of intermediate lobe pro-opiomelanocortin after short- and long-term stress suggest that (i) specific intermediate lobe products are differentially released after stress; (ii) repeated activation leads to an induction of biosynthesis of pro-opiomelanocortin, resulting in increased conversion from precursor to products and increased production of N-acetylated β-endorphin-(1–31) [*N*Ac β-endorphin-(1–31)]; (iii) the increase in biosynthesis is accompanied by increased release of *N*Ac β-endorphin-(1–31) and α-MSH; and (iv) there is no evident way to predict which peptide is likely to be selectively regulated and released, as *N*Ac β-endorphin-(1–31) is neither the most active nor the most dominant stored form.

Recently, Przewlocki *et al.* (*4*) showed that the intermediate lobe can be activated by short-term stress. We examined the effect of both a single stress and phasically repeated stress on the regulation of pro-opiomelanocortin. The stress procedure, identical to the one that produces stress-induced analgesia (*5*), consisted of 30-minute sessions of intermittent footshock. Four groups of rats were studied in each experiment: (i) a control group, which was not stressed; (ii) a group whose members were stressed on a single occasion immediately before being killed (acutely stressed group); (iii) a repeatedly stressed group whose members received 30 minutes of daily footshock and were then allowed to rest for 24 hours before being killed (chronically stressed/rested group); and (iv) a repeatedly stressed group whose members were stressed daily for 2 weeks

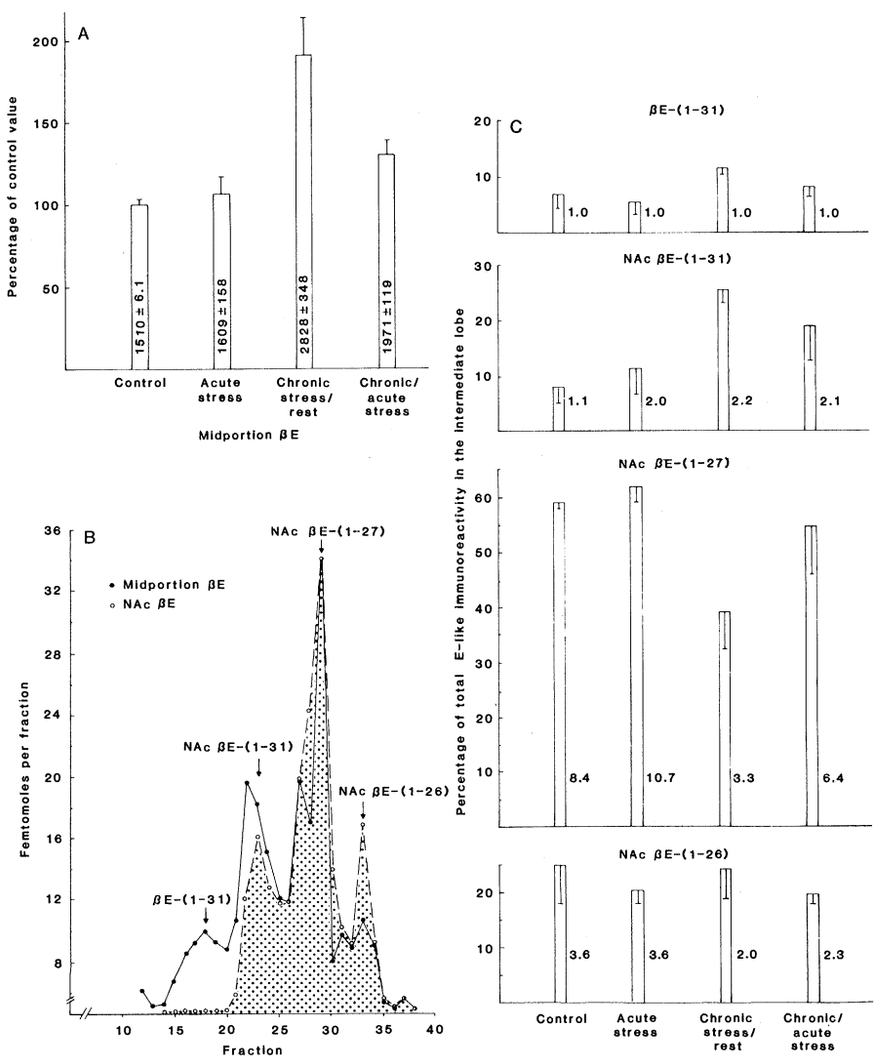

Fig. 1. Effect of acute and chronic stress on the content and forms of β-endorphin (βE) in the intermediate lobe. (A) Total βE-like immunoreactivity across the four experimental groups. Immunoreactivity was a significantly elevated ($P < 0.01$) in the chronically stressed and rested group. (B) Typical HPLC profile of the intermediate lobe in acutely stressed animals. We used a reversed-phase system on a C-18 octadecyl silane Ultrasphere column with 40 m*M* potassium phosphate and 0.1 percent triethylamine as solvent A and acetonitrile: methanol (9:1) as solvent B. The gradient rose from 25 to 45 percent in 30 minutes (flow rate, 1 ml/min; fraction size, 0.5 ml). Fractions were collected, evaporated, and assayed with a midportion antibody that recognizes all βE forms (solid lines) and an *N*-acetyl–specific antiserum that requires α-NH_2-tyrosine acetylation (dashed lines, dotted profiles). (C) Relative amounts of βE-(1–31), *N*Ac βE-(1–31), *N*Ac βE-(1–27), *N*Ac βE-(1–26) (major peaks on HPLC) under the four stress conditions. Note that βE-(1–31) and *N*Ac βE-(1–31) increase while *N*Ac βE-(1–27) decreases with stress followed by rest. These changes are reversed with chronic, followed by acute, stress. The numbers beside the bars indicate the relative proportions of the peptides in each group, with βE-(1–31) taken as 1.

and restressed immediately before decapitation (chronically stressed/acutely stressed group).

To measure β-endorphin we used an antiserum to the midportion of this polypeptide, which thus reacts with all its forms, and an antibody to *N*Ac β-endorphin, which reacts only with forms having acetyl groups at the amino terminus (that is, specific products of the intermediate lobe). We could then deduce the relative concentrations of nonacetylated and acetylated β-endorphin–like peptides. The antiserum to α-MSH is specific for the carboxyl terminus and thus can react with des-acetyl, mono-acetyl, and diacetyl α-MSH.

In the first experiment (eight animals per group), we examined the effect of the four manipulations on *N*Ac β-endorphin-like material in plasma. In the control group we detected 32.3 ± 6.6 fmol of such material per milliliter. In the acutely stressed group there was a small but reliable increase to 54.0 ± 7.1 fmol/ml. In the chronically stressed and rested animals a return to the control concentration was observed (31.4 ± 6.5 fmol/ml). However, the animals that were chronically and acutely stressed showed a substantial increase (132 ± 11 fmol/ml). This increase in release from the intermediate lobe was confirmed by determining the β-endorphin content of the pituitaries (Fig. 1A). No significant depletion of content followed a single stress. Repeated stress led to a substantial accumulation of β-endorphin–like material in the gland. The accumulated material was apparently highly releasable, as evidenced by a significant decline in its content in the intermediate lobes of the chronically and acutely stressed group (Fig. 1A).

We then examined the question of how the forms of β-endorphin were altered in the intermediate lobe under the various conditions. In two separate experiments (eight animals per experiment) the forms of β-endorphin in each of the four groups were separated by high-performance liquid chromatography (HPLC) coupled to midportion and *N*-acetyl–specific radioimmunoassays (Fig. 1B). The four dominant forms were β-endorphin-(1–31) and *N*Ac β-endorphins (1–31), (1–27), and (1–26). The results of the two HPLC studies were averaged and show that the profiles of the β-endorphins are different in the four groups (Fig. 1C). With repeated stress followed by 24 hours of rest, β-endorphin-(1–31) and *N*Ac β-endorphin-(1–31) accumulated at the expense of *N*Ac β-endorphin-(1–27). Thus in the control rats the ratio of β-endorphin-(1–31):*N*Ac β-endorphin-(1–31):*N*Ac β-endorphin-(1–27):*N*Ac β-endorphin-(1–26) was 1:1.1:8.4:3.6. It was 1:2.2:3.3:3.6 in the chronically stressed and rested group. *N*Ac β-endorphin-(1–27) usually accounted for 60 percent of the total immunoreactivity. In chronically stressed and rested animals it accounted for less than 40 percent. This relative accumulation of the larger forms at the expense of *N*Ac β-endorphin-(1–27) was partially reversed when the animals were restressed before decapitation (chronically and acutely stressed group). After that treatment, *N*Ac β-endorphin-(1–27) represented 55 percent of the total β-endorphin content. This change was due to selective release of β-endorphin and *N*Ac β-endorphin-(1–31) or to increased cleavage of β-endorphin at the carboxyl terminus.

These results suggested that *N*Ac β-endorphin-(1–31) was highly sensitive to the stress manipulation. Was it being selectively released? Molecular sizing of the plasma confirmed that this form was indeed selectively liberated in the chronically and acutely stressed group (Fig. 2). It is difficult to ascertain whether the intermediate lobe also releases unmodified β-endorphin-(1–31) into plasma, since this form is also derived from the anterior lobe. The release of α-MSH (another index of intermediate lobe activation) also increased in the same group (Fig. 2). Whether acetylated or des-acetylated α-MSH was being released will require further analysis by HPLC.

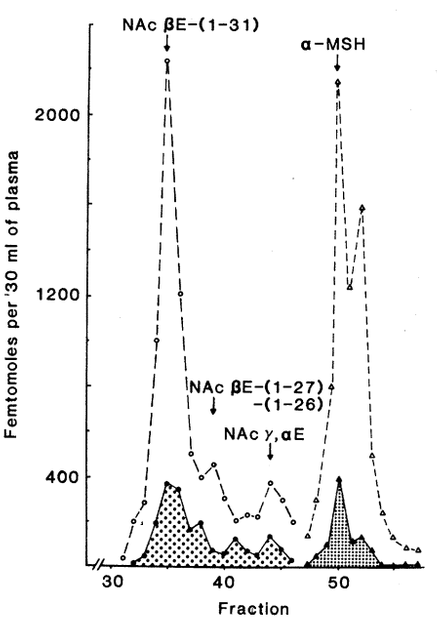

Fig. 2. Plasma profiles of intermediate-lobe products, as determined on Sephadex G-50 in 1 percent formic acid (fraction size, 2 ml; column size, 1.5 by 90 cm). Symbols: (○) *N*Ac βE in the chronically and acutely stressed group; (●) *N*Ac in the acutely stressed group; (△) α-MSH in the chronically and acutely stressed group; and (▲) α-MSH in the acutely stressed group.

Since *N*Ac β-endorphin-(1–31) requires β-endorphin formation followed by *N*-acetylation, its accumulation suggested that the enzymes up to that point in the biosynthetic pathway must have been activated. Support for this idea came from biosynthetic data from primary cultures of intermediate lobes from the four groups. The procedures used in preparing these short-lived cultures are described in the legend to Fig. 3. The intermediate lobe cells were cultured immediately after stress treatment and decapitation to preserve the biosynthetic changes induced in vivo. In acutely stressed rats there was little change in the pattern of β-endorphin biosynthesis (the half-life of pro-opiomelanocortin was 30 minutes, compared to 27.3 minutes for control animals). However, chronically stressed and rested animals exhibited a slight increase of incorporation of [^{3}H]lysine into pro-opiomelanocortin and a clear change in conversion of pro-opiomelanocortin to β-lipotropin and β-endorphin (half-life of pro-opiomelanocortin, 21.7 minutes). The biosynthetic rate increased even more markedly in the chronically and acutely stressed rats (16.7 minutes). Thus the half-life of pro-opiomelanocortin after both chronic and acute stress was half that seen after acute stress only (Fig. 3C). These findings support the HPLC results. It appears that β-endorphin processing rates are increased by repeated stress and that substantial release is followed by increased biosynthesis.

In sum, we have found that (i) the intermediate lobe releases its products (*N*Ac β-endorphin and α-MSH) into the bloodstream more readily after repeated stress; (ii) *N*Ac β-endorphin-(1–31) accumulates with stress and appears as the dominant form in plasma, apparently because of selective release; and (iii) with repeated activation there is induction of biosynthesis and acceleration of processing, as shown by the primary culture studies and HPLC data.

The mechanism by which repeated stress induces the intermediate lobe is unclear. It was recently reported (*6*) that the stress-induced release is controlled by β-adrenoreceptor mechanisms. Repeated stress induces the adrenal medulla, leading to greater release of catecholamines. This in turn may induce biosynthesis of pro-opiomelanocortin in the intermediate lobe. That we found a 50 to 60 percent increase in the rate of lysine incorporation into the precursor and greater changes (doubling) in the speed of conversion from precursor to product

suggests the importance of the enzymes responsible for maturation of the precursor. The fact that *N*Ac β-endorphins (1–31) and (1–26) accumulate in chronically stressed and rested rats, while *N*Ac β-endorphin-(1–27) is relatively decreased, supports the idea that enzymatic cleavage of the carboxyl terminus may constitute a rate-limiting step along the biosynthetic pathway (*2*) and that *N*-acetylation and histidine removal are not as rate-limiting.

Our results suggest that there may not be an a priori method to predetermine the concentrations of biosynthetic intermediates, products, and metabolites when multiple posttranslationally modified forms are stored together. *N*Ac β-endorphin-(1–31), which is selectively released and selectively accumulated on stimulation of the intermediate lobe, is neither the most biologically active form at the opioid receptors nor the most dominant stored form. It is possible that, while devoid of opioid activity (*3*), it has other biological effects. Such nonopioid effects of endorphins have been demonstrated for β-endorphin and dynorphin (*7*). More important, its regulation appears to be mediated by multiple enzymatic events that occur at various rates and that remain to be fully described.

While *N*Ac β-endorphin-(1–31) is the most releasable and most regulated form secreted by the intermediate lobe, other results from our laboratory show that the anterior lobe readily releases β-endorphin-(1–31) rather than lipotropin in response to stress or corticotropin-releasing factor. Simultaneously, β-endorphin-(1–31) in the anterior lobe is the form most subject to differential induction and regulation. Possibly a hallmark of a "true" product is not its abundance, but its selective release or differential regulation in response to the appropriate stimuli.

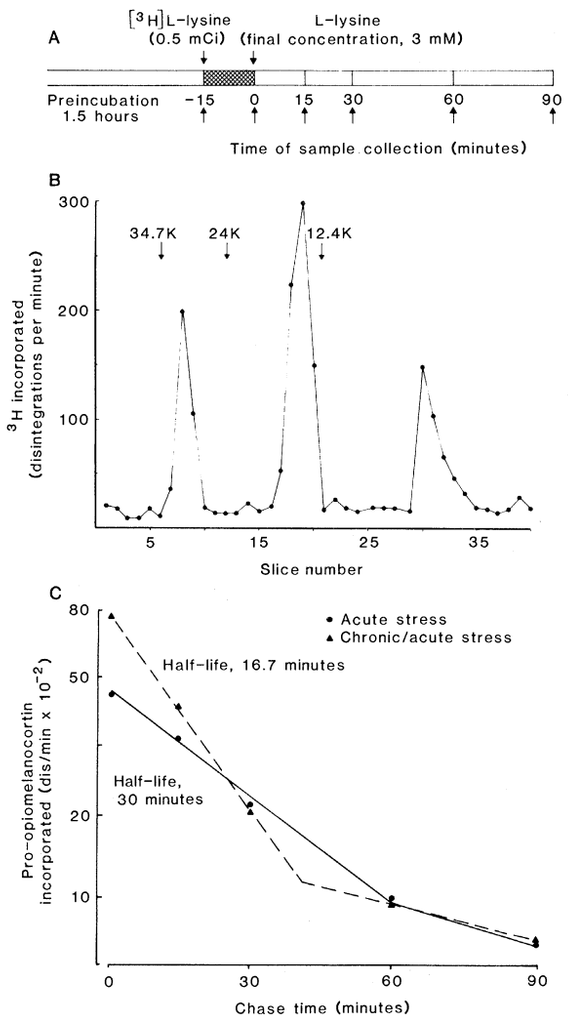

Fig. 3. Effects of stress on biosynthetic rate in short-term cultures of rat intermediate lobes established immediately after the animals were killed. Pituitaries were divided into anterior and intermediate lobes and immersed in oxygenated Krebs-Ringer-bicarbonate medium (KRB) containing crude collagenase (3 mg/ml; Sigma type 1) and bovine serum albumin (5 mg/ml; Sigma fraction 5) and incubated in an atmosphere of 5 percent CO_2 and 95 percent O_2 for 1 hour at 37°C. The cells were then rinsed three times with KRB containing kanamycin sulfate (0.1 mg/ml), soybean trypsin inhibitor (0.1 mg/ml), lima bean trypsin inhibitor (0.1 mg/ml), aprotinin (50 IU/ml), and bovine serum albumin (5 mg/ml). The cells were transferred to incubation vials in a volume of 200 μl and incubated for 1.5 hours at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2. Each sample yielded 5×10^4 cells per intermediate lobe. Viability, as determined with the trypan blue exclusion technique, was 95 percent. After incubation, 0.5 mCi of [^{3}H]lysine (specific activity, 65 Ci/mmol; New England Nuclear) was added to each vial in a volume of 0.05 ml of KRB to label the proteins being synthesized. This labeling period lasted 15 minutes. Labeling was terminated by the addition of unlabeled lysine (20 m*M*) and the samples were further incubated for 15, 30, 60, or 90 minutes (chase period). After the chase period each sample was washed three times with KRB containing 20 m*M* unlabeled L-lysine and then extracted with 0.5 ml of ice-cold 2*N* acetic acid containing iodoacetamide (0.3 mg/ml) and phenylmethylsulfonyl fluoride. Incorporation of labeled amino acids into proteins was measured by precipitation in trichloroacetic acid. The extracts were lyophilized, dissolved in 0.3 ml of 150 m*M* sodium phosphate buffer (*p*H 8.2) containing 0.3 percent bovine serum albumin, and then applied to a column containing antibody to β-endorphin that had been equilibrated with 10 ml of the same buffer. After 30 minutes of incubation the column was washed with 30 ml of 150 m*M* potassium phosphate buffer (*p*H 8.2) followed by 150 m*M* pyridine solution. Captured materials were eluted with 5 ml of 2*N* acetic acid and lyophilized. Recovery was 92 percent. The purified samples were separated on sodium dodecyl sulfate–polyacrylamide gels (percent crosslinkage, 12.5 percent gel). All samples contained dansylated pepsin (39.7K), dansylated trypsinogen (24K), and dansylated cytochrome c (12.4K) as internal standards. After electrophoresis, gels were cut into 2-mm-thick disks and the slices were eluted in 0.5 ml of 50 m*M* sodium phosphate buffer (*p*H 7.6) containing 0.06 percent SDS and bovine serum albumin (0.2 mg/ml) at 37°C for 16 hours (recovery, 90 to 95 percent) and then counted. (A) Time course of the pulse-chase experiment. (B) Sodium dodecyl sulfate gel profile of the immunoaffinity-purified material at 30 minutes of chase, showing a 31K-sized pro-opiomelanocortin peak, as well as a β-lipotropin–sized and β-endorphin–sized peak. Such gels at each time point for each group allowed computation of incorporation into pro-opiomelanocortin and of the rate of pro-opiomelanocortin disappearance and conversion into β-lipotropin and β-endorphin. (C) Rates of disappearance of pro-opiomelanocortin upon chase in the acutely stressed group and the chronically and acutely stressed group.

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