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In Vitro Release of Hypothalamic β-Endorphin (βE) by Arginine Vasopressin, Corticotropin-Releasing Hormone and 5-Hydroxytryptamine: Evidence for Release of Opioid Active and Inactive βE Forms

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Abstract—The aims of the present experiments were: 1) to test whether substances which modulate beta-endorphin-immunoreactive (βE-ir) release from the pituitary gland might act similarly in hypothalamic tissue; and 2) to further characterize the βE-ir forms which are released from hypothalamus. To address these questions, hypothalamic tissue was incubated *in vitro* for 10 min periods in either normal media (basal conditions) or in media containing 55 mM KCl or one of several other test substances (stimulation conditions) and release was estimated by measuring the βE-ir concentrations in the media. Depolarizing concentrations of K^+ increased βE-ir release 2-3 fold over basal levels and this effect appeared to be Ca^{2+}-dependent. Dose-dependent increases in βE-ir release were elicited by nanonolar to micromolar concentrations of either corticotropin-releasing hormone (CRH), arginine vasopressin (AVP), or 5-hydroxytryptamine (5-HT). Conversely, dopamine (1 μM) inhibited both the basal and K^+-stimulated release of βE-ir from hypothalamus. Gel filtration chromatography revealed that $βE_{1-31}$ and $βE_{1-27}/βE_{1-26}$ were the primary βE-ir peptides released under either basal or CRH-stimulated conditions; the relative amounts of the βE-ir peptides found in the media were nearly identical to those found in the hypothalamus itself. This result indicates that the release of different βE-ir peptides into the media appears to be proportional to the relative amounts stored in tissue. In light of the critical roles of CRH and AVP in mediating stress responses in the anterior pituitary, the data showing that the same neurohormones/transmitters (i.e., CRH, AVP, 5-HT, and DA) modulate βE-ir release in both the pituitary gland and the hypothalamus suggests that endocrine and central POMC systems involved in stress responses might be regulated in a coordinate manner.

Introduction

Beta-endorphin (βE)-containing pathways in the central nervous system are believed to be involved in a number of behavioural responses (e.g., stress,

nociception). However, it has been difficult to determine the precise neurochemical and neuro-anatomical mechanisms which mediate these responses. The major pro-opiomelanocortin (POMC) cell group is localized in the arcuate nucleus of the hypothalamus with diverse projections to forebrain and midbrain areas including the

septum, amygdala, the periventricular nucleus of the thalamus, various hypothalamic nuclei, and the diencephalic/midbrain central gray region (1). In all brain regions studied to date, βE_{1-31}, βE_{1-27}, and βE_{1-26} are the predominant POMC products detected; except for the brainstem, only a small percentage of the βE-ir peptides in brain exist in acetylated forms (2-5).

While the anatomy and biochemistry of brain POMC systems are relatively well characterized, the neurochemical modulators and the mechanisms of biosynthetic regulation in these neurons are largely unknown. Initially, putative neuroregulators of βE-immunoreactive (βE-ir) release in brain were inferred from alterations in steady-state βE-ir concentrations following pharmacological manipulations. However, because of the non-specific nature of *in vivo* pharmacological treatments and the complexity of neural systems, researchers have used *in vitro* paradigms to study what modulates βE release from brain tissue. Results from *in vitro* studies have demonstrated that depolarizing concentrations of K^+ increase βE-ir release from hypothalamic tissue in a Ca^{2+}-dependent manner (6-9). Corticotropin-releasing hormone (CRH) has also been shown to elicit βE-ir release and this effect is blocked by the addition of the CRH receptor antagonist, α-helical CRH_{9-41} (10). Dopamine (DA) has been reported to increase βE-ir release from human hypothalamus (11) but to decrease release from rat hypothalamus (7). Since CRH and DA are both potent regulators of βE-ir release in the pituitary gland, one of the aims of the present studies was to examine whether other pituitary secretagogues might also alter βE-ir release from hypothalamic tissue.

It has previously been shown that secretagogues release predominantly βE_{1-31}-size material into the media from hypothalamic tissue (8, 11), although there have also been reports that β-LPH-size peptides represent as much as 25-50% of the total βE-ir released (6, 10). However, those chromatographic analyses did not determine whether this material was authentic βE_{1-31} or might be carboxy-terminal cleaved products of βE_{1-31}, i.e., βE_{1-27} or βE_{1-26}. From a physiological standpoint, this issue could be extremely important since βE_{1-27} and βE_{1-26} are devoid of (12, 13),

or are antagonistic to (14, 15), the opiate properties of full length βE_{1-31}. The precise nature of a cellular response would depend upon the relative amounts of opiate-active versus opiate-inactive βE-ir material released at a synapse. However, since evidence in both the anterior (16) and intermediate (17) lobes of the pituitary gland indicates that the βE-ir peptides stored in a tissue do not necessarily predict which peptides are released from that tissue, a second aim of these studies was to determine the βE-ir species which are secreted into the media from the hypothalamus.

Methods

Animals

Male Sprague-Dawley rats (Charles River Co., Wilmington, MA), weighing 220-250 gm at the start of the experiments, were maintained in groups of 5-6 per cage, with free access to food and water, in an environmentally controlled room (12:12 hr light:dark cycle, lights on at 0600 hr). Animals were sacrificed by decapitation between 0900 and 1100 hr and hypothalami were dissected on ice. Hypothalamic blocks, weighing 40-60 mg, were defined by the optic chiasm, the mammilary bodies, the lateral hypothalamic fissures, and the top of the 3rd ventricle.

In vitro *release*

Hypothalamic blocks were sliced into 5-6 pieces and placed individually or in pairs in plastic carriers (13 mm diam.) with nylon mesh on the bottom to support the tissue. Carriers were placed in tray wells (Costar) with 800 μl of Krebs-Ringer-Bicarbonate (KRB) buffer (120 mM NaCl, 5 mM KCl, 27.5 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 2.6 mM $CaCl_2$, 0.7 mM $MgSO_4$) containing 0.1% BSA, 0.18% glucose, and 0.03% bacitracin. Tissue was equilibrated in this media at 37°C with O_2:CO_2 (95.5%) for approximately 50 min. After the equilibration period, tissues were transferred every 10 min to new wells containing either normal KRB buffer, a high concentration (55 mM KCl) K^+-KRB buffer, or normal KRB buffer containing a test chemical(s). The K^+-KRB buffer was made

isomolar with the normal KRB buffer by decreasing the NaCl concentration to 70 mM. Ca^{2+}-free media was prepared by omitting $CaCl_2$ and adding 1 mM EGTA. At the end of each incubation period, the buffer was collected and assayed directly by RIA to determine the amount of βE-ir that had been released into the media.

Drugs. Carbachol, dopamine HCl (DA), 5-hydroxytryptamine HCl (5-HT) were obtained from Sigma (St. Louis, MO); CRH and Arg^8-vasopressin (AVP) were from Bachem (Torrance, CA).

Peptide quantitation

βE-ir was measured by a radioimmunoassay using an antibody directed primarily against the mid-portion of $βE_{1-31}$ (i.e., $βE_{17-27}$). At the concentration used in this assay (1:40000), and using ^{125}I N-acetyl-$βE_{1-27}$ as the radiolabelled tracer, the antibody was completely crossreactive with β-LPH, $β_{1-31}$, $βE_{1-27}$, $βE_{1-26}$ and their N-acetlated derivatives. The antibody did not crossreact with $βE_{1-16}$, $βE_{1-17}$, des-tyrosine $βE_{1-17}$, $βE_{27-31}$, $ACTH_{1-39}$, αMSH or γMSH or with peptides from other opioid precursors (e.g., Leu-enkephalin, Met-enkephalin, dynorphin A, dynorphin B, α-neo-endorphin). Sensitivity of the assay under disequilibrium conditions was 1-3 fmoles per tube, with an IC_{50} of approximately 13 fmoles. Tissue samples and chromatographic fractions were quantitated against a camel $βE_{1-31}$ standard curve. Hypothalamic tissue was homogenized in acetone:0.2N HCl (3:1), the supernatant was dried down and aliquots were resuspended in RIA buffer (150 mM Na phosphate buffer with 1% NaCl and 0.1% bovine serum albumin, pH 8.2). Media were assayed directly and were quantitated relative to a camel $βE_{1-31}$ standard curve dissolved in KRB buffer.

Gel filtration. Different sized βE-ir forms (e.g., $βE_{1-31}$, $βE_{1-26/1-27}$) were determined by chromatography on a Sephadex G50-50 (1.5 × 90 cm) column developed with 1% formic acid containing 0.01% BSA. When media were analyzed, 6-9 fractions of basal or CRH-stimulated fractions were pooled. Media pools or hypothalamic extracts were dried down and loaded on the column in 1% formic acid containing 0.01% BSA. Collected fractions (1.3 ml) were vacuum dried,

resuspended in RIA buffer, and assayed for βE-ir content. The column was pre-calibrated with dextran blue, camel $βE_{1-31}$, camel $βE_{1-27}$, and cobalt chloride.

Statistics

Stimulated βE-ir release was defined as the amount of βE-ir released by a test chemical minus the basal release. Basal release was calculated as the average amount of βE-ir released in normal KRB buffer in the fraction immediately preceding and the 2 fractions following the test chemical incubation. Differences between treatment groups were statistically evaluated by t-tests or one way ANOVAs followed by Dunnett t-tests (significance level of $p < 0.05$).
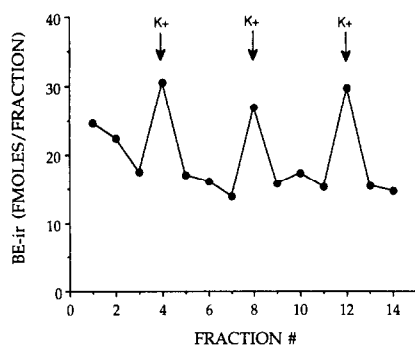
Results

In establishing the *in vitro* release system, we were concerned about the possible loss of βE-ir due to enzymatic degradation and/or to adsorption (e.g., to the carrier or wells, or to the brain tissue itself). To address this issue, hypothalamic tissue was incubated in K^+-KRB buffer containing ^3H-$βE_{1-31}$ (150000 CPM; courtesy of the late Dr C. H. Li) for either 7.5 or 30 min. Following the incubation, media was chromatographed on a Sephadex G-50-50 column and the eluted radioactivity was counted on a beta-counter. Virtually all of the radioactive βE that was incubated in media for 7.5 min was recovered in a single peak that corresponded to the elution position of authentic $β_{1-31}$ (Fig. 1). Even after 30 min, 87% of the radioactive βE added appeared to be intact $βE_{1-31}$, with the remaining radioactivity eluting at a molecular weight of 1700, the approximate elution points of $βE_{1-16}$ (α-endorphin) or $βE_{117}$ (γ-endorphin). No peak co-eluting with $βE_{1-27}$ was observed. These data indicate that, for the short duration incubations we used in these experiments, recovery of exogenously added βE was excellent and proteolysis of βE was minimal.

High concentrations of KCl increased βE release from hypothalamic blocks *in vitro*, as 2-3 fold higher levels of βE-ir were detected when tissue was incubated in K^+-KRB buffer compared to levels found in normal KRB buffer (i.e., basal
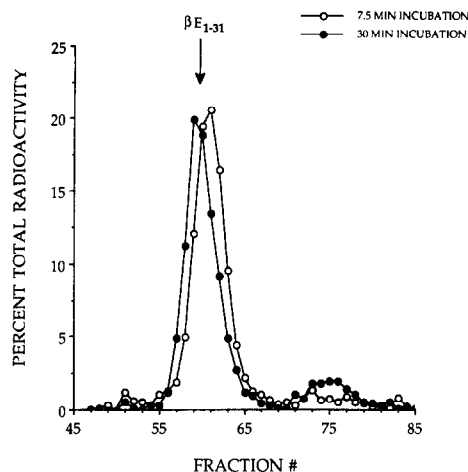
Fig. 1 Recovery of ^{3}H-βE_{1-31} from media following incubation with hypothalamic tissue. 150000 CPM of ^{3}H-βE_{1-31} (courtesy of the late Dr C. H. Li) was incubated in 55mM K$^+$-KRB buffer with hypothalamic tissue for either 7.5 or 30 min. Media was chromatographed on a Sephadex G-50-50 column with 1.0% formic acid containing 0.01% BSA and radioactivity in collected fractions was counted in a liquid scintillation counter. The CPM per fraction are expressed as a percentage of the total CPM added to the media. The elution position of synthetic camel βE_{1-31} is indicated.

release). K$^+$-stimulated release was reliably elicited multiple times over several hours of incubation (Fig. 2). In studies assessing the affect of Ca^{2+} removal on βE-ir release, we found that basal βE-ir release was unaffected but K$^+$-stimulated release was greatly reduced in Ca^{2+}-free

media (data not shown). It should be noted that K$^+$-induced βE-ir release was only partially inhibited when hypothalami were first incubated in normal KRB before being placed in Ca^{2+}-free media, whereas Ca^{2+} removal completely blocked K$^+$-induced release when hypothalami were first incubated in Ca^{2+}-free media.

Different chemicals tested in these studies increased, decreased or had no effect on βE-ir levels found in the media. Dose-dependent increases in βE-ir release were induced by CRH, AVP, and 5-HT (Fig. 3), with AVP and 5-HT being the most and least potent secretagogues, respectively. DA inhibited both basal and K$^+$-stimulated release of βE from hypothalamus (Fig. 4) while carbachol had no effect on βE release (data not shown).

Chromatographic analyses of media collected following incubation in normal KRB or normal KRB containing 100 nM CRF revealed that there were 2 major peaks of βE-ir, the larger peak co-eluting with the βE_{1-31} standard and the second peak eluting with $\beta E_{1-27}/\beta E_{1-26}$ (which could not be separated on this column; Fig. 5a). The relative amounts of βE_{1-31} and $\beta E_{1-27}/\beta E_{1-26}$ found in media under basal and stimulated conditions did not differ significantly (1.1:1 and 1.3:1, respectively). Furthermore, the peptide forms of βE found in media were nearly identical to those found in hypothalamic tissue ($\beta E_{1-31}:\beta E_{1-27}/\beta E_{1-26}$ ratio = 1.3:1; Fig. 5b).
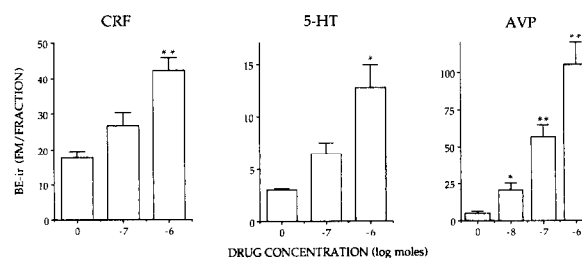

Fig. 2 A typical experiment demonstrating basal and K$^+$-stimulated βE-ir release from hypothalamus. Hypothalamic tissue was incubated for 10 min periods in either normal KRB buffer or in 55mM K$^+$-KRB buffer (indicated by the arrows). Media was frozen immediately following incubation and later assayed directly for βE-ir. The data are expressed as fmoles of βE-ir detected per 10 min incubation period.

Fig. 3 Dose-dependent release of βE-ir from hypothalamus by CRH, AVP and 5-HT. Hypothalamic tissue was incubated for 10 min periods in either normal KRB buffer or in normal KRB containing different concentrations of CRH, AVP or 5-HT. βE-ir in the media was measured by RIA and is expressed as fmoles βE-ir per 10 min incubation period. Data represents the means ± S.E.M. of 2-8 different incubation periods. * p < 0.01, ** p < 0.001 compared to control (no drug) group.
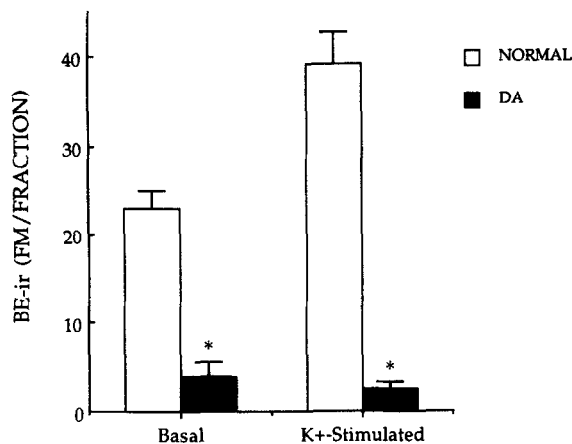
Fig. 4 Inhibition of basal and K^+-stimulated βE-ir release by dopamine. Hypothalamic tissue was incubated in normal and 55 mM K^+-KRB buffers in the absence (Normal) or presence of 10^{-6} M dopamine (DA) and βE-ir was measured in the media. Data are expressed as fmoles βE-ir per 10 min incubation period and represent the means ± S.E.M. of 4 different chambers. * $p < 0.001$ compared to normal group.

Discussion

The two main findings in these studies were: 1) in vitro release of βE from hypothalamus was stimulated in a dose-dependent manner by CRH, AVP, and 5-HT and was inhibited by DA; and 2) the relative amounts of $βE_{1-31}$ and $βE_{1-27}/βE_{1-26}$ released into the incubation media, under either basal or stimulated conditions, mirrored βE-ir forms found in hypothalamic tissue. These results characterizing βE-ir release from the hypothalamus will be discussed in relation to the regulation of βE-ir release from the pituitary gland.

In these studies, we demonstrated that 55 mM K^+, as well as a number of putative neurotransmitters, increased the secretion of βE-ir into the incubation buffer. That this increase reflects stimulation-induced release of βE-ir from hypothalamic tissue is supported by the fact that release appears to be Ca^{2+}-dependent, i.e., depolarization-induced Ca^{2+} influx appears necessary for release. Furthermore, the similarity between the relative amounts of $βE_{1-31}$ and $βE_{1-27}/βE_{1-26}$ found in hypothalamus and media suggests that the βE-ir peptides found in the media are most likely derived by release from intracellular pools. While we cannot exclude the possibility

that the βE-ir forms detected in the media are proteolytically derived from larger βE-ir peptides released from tissue, our data showing minimal degradation of exogenously added ^3H-βE (see Fig. 1) and the fact that larger βE-ir peptides are not stored in the hypothalamus argue against this possibility. Rather, we believe these data indicate that the βE-ir peptides released from hypothalamic POMC neurons in vitro reflect the proportional amounts stored within these cells.

In contrast to the present data in the hypothalamus, there is evidence that proportional release of stored βE-ir species does not always occur in either the anterior or intermediate lobes of the pituitary gland (16-19). For example, whereas approximately twice as much β-lipoprotein (β-LPH) as $βE_{1-31}$ is stored in anterior lobe corticotrophs, roughly 2-fold more $βE_{1-31}$ than β-LPH is released by an acute stressor in vivo or by CRH in vitro (16). One explanation for the discrepancy between the peptides that a cell stores and those it releases is that the extent of POMC processing may not be the same in all vesicles within an individual cell; vesicles containing more processed βE-ir species may accumulate closer to the outer membrane,
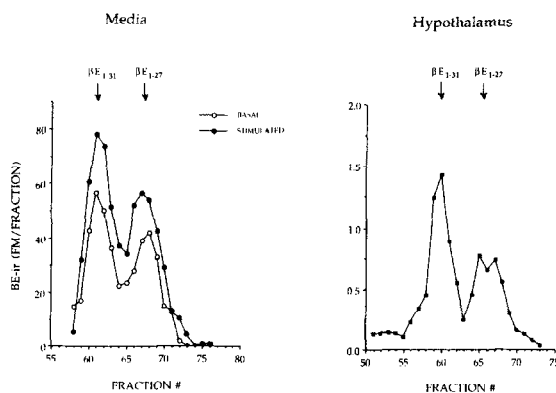
Fig. 5 Different sized βE-ir peptides found in a) media and b) hypothalamus. Hypothalamic tissue was incubated in normal KRB buffer or normal KRB containing 10^{-6} M CRH. Media from basal and CRH-stimulated fractions were pooled and hypothalamic tissue was extracted in acetone:0.1 N HCl (3:1). a) Media pools or b) hypothalamic extracts were developed on a Sephadex G-50-50 column with 1.0% formic acid containing 0.01% BSA and βE-ir was measured in collected fractions by RIA. Data are expressed as a) fmoles or b) pmoles of βE-ir per fraction. The elution positions of synthetic camel $βE_{1-31}$ and $βE_{1-27}$ are indicated.

making these pools more releasable. A second possibility is that subpopulations of corticotrophs in the pituitary, storing different mixtures of POMC-processed peptides, have different sensitivities to secretagogues such as CRH or AVP. Since the various βE-ir peptide products produce extremely different behavioural and physiological effects (e.g., N-acetylation or carboxy-terminal cleavage of βE_{1-31} result in products which are much weaker opioid agonists than the original peptide (12, 13, 15)), the possibility that cells could selectively release a particular peptide product under different conditions is extremely exciting. However, under the conditions tested in the present experiments, hypothalamic POMC cells did not exhibit this property. It would be interesting to determine whether proportional βE-ir release from hypothalamic tissue would still occur if the relative amounts of βE_{1-31} and βE_{1-27} stored in cells were experimentally altered.

An important question to address is whether the stimulatory effects of CRH, AVP and 5-HT on βER-ir release are mediated directly at POMC cells or indirectly by modulating transmitter release from other neurons which synapse upon POMC cells. Since binding sites for CRH (20), AVP (21), and 5-HT (22) have been demonstrated in the hypothalamus, it is possible that POMC cells possess each type of these receptors and can thus be directly stimulated by these secretagogues. However, until these different receptor types are localized to POMC-containing cells in particular, it will be difficult to ascertain whether some or all of these transmitters are acting directly or whether one of these substances might act as a final common transmitter in modulating βE release. It is interesting that in the hypothalamus, AVP appears to be approximately 10 fold more potent than CRH in stimulating βE-ir release, whereas the converse is true in the anterior pituitary either in vivo (28) or in vitro (29). One reason why CRH appears to be a less potent βE secretagogue than AVP in the hypothalamus might be because it is acting via other neurons to stimulate βE release while AVP stimulates secretion directly from POMC neurons. A second explanation for the reversed sensitivities to CRH and AVP may be related to the relative distribution of the receptors for these peptides in brain and pituitary POMC

cells. For example, if the majority of corticotrophs possess CRH receptors and the remainder of cells contain AVP receptors, then CRH could appear to be a more potent βE secretagogue than AVP; conversely, the majority of hypothalamic POMC neurons may contain AVP receptors, making this tissue more sensitive to AVP relative to CRH. Detailed neuroanatomical mapping of the hypothalamus, in conjuction with immunohistochemistry and receptor autoradiography, will aid in resolving which, if any, of these explanations are correct. The fact that there is a rich supply of CRH, AVP, and 5-HT nerve terminals (30, 31) and binding sites (20-22) in the hypothalamus supports the possiblity that CRH, AVP, and 5-HT have physiological effects similar to the pharmacological ones observed here.

Addition of DA to the incubation media inhibited both basal and K^+-stimulated release of βE-ir from hypothalamus. The mechanism by which DA inhibits either basal or stimulated βE-ir release from the hypothalamus is presently not known. In fact, it is unclear what the βE-ir found in the media under basal conditions actually represents. While it may reflect the basal rate of release from spontaneously discharging neurons, it may also represent, at least in part, peptide that has been discharged from dying cells. However, the fact that DA significantly inhibited basal release suggests that tonic βE release may be an active mechanism which can be modulated by inhibitory neurotransmitters.

The most intriguing aspect of the present results is that each of the substances which modulate βE-ir release from hypothalamic tissue (i.e., CRH, AVP, 5-HT, DA) have previously been shown to produce similar effects on ACTH and βE release in the anterior pituitary gland (23-27). Given the functional importance of CRH and AVP in mediating stress responses in the pituitary, it is reasonable to speculate that these agents might also play a role in stress responses in the central nervous system. There is evidence to indicate that brain opioid systems, in particular POMC systems, are involved in stress responses. For example, there is a significant decrease in βE-ir in the hypothalamus after footschock stress (32-33). Following repeated exposure to this stressor, midbrain concentrations of βE-ir peptides and hypo-

thalamic POMC mRNA levels increase, suggesting that POMC biosynthesis has accelerated in response to the increased drive upon this system (Akil et al., in preparation). The stress-induced changes in POMC peptides and mRNA observed in the brain follow similar patterns to those seen in the anterior pituitary gland (34). Although the roles of CRH and/or AVP in producing stress-induced changes in POMC systems in the brain have not been established (as they have in the anterior pituitary), the present data suggest the possibility that the same transmitters (i.e., CRH, AVP and perhaps 5-HT and DA) may be involved in regulating the two higher levels of the hypothalamo-pituitary-adrenal (HPA) axis. Regulation of βE-ir release in both the pituitary and hypothalamus by common neurohormones/transmitters may represent a mechanism by which organisms coordinate central and endocrine responses to stress.

Acknowledgements

References

1. Khachaturian, H., Lewis, M. E., Schafer, M.K.-H. and Watson, S. J. (1985). Anatomy of the CNS opioid systems. Trends in Neuroscience 8: 111-119.
2. Zakarian, S. and Smythe, D. G. (1982). Beta-endorphin is processed differently in specific regions of rat pituitary and brain. Nature (London) 296: 250-252.
3. Dores, R. M., Jain, M., and Akil, H. (1986). Characterization of the forms of β-endorphin and α-MSH in the caudal medulla of the rat and guinea pig. Brain Res. 377: 251-260.
4. Emeson, R. B. and Eipper, B. A. (1986). Characterization of pro-ACTH/endorphin-derived peptides in rat hypothalamus. J. Neurosci. 6: 837-849.
5. Weber, E., Evans, C. J. and Barchas, J. D. (1981). Acetylated and nonacetylated forms of beta-endorphin in rat brain and pituitary. Biochem. Biophys. Res. Commun. 103: 698-705.
6. Vermes, I., Mulder, G. H., Berkenbosch, F. and Tilders, F. J. H. (1981). Release of β-lipotropin and β-endorphin from rat hypothalami in vitro. Brain Res. 211: 248-254.30.

7. Vermes, I., Tilders, F. J. H. and Stoof, J. C. (1985). Dopamine inhibits the release of immunoreactive β-endorphin from rat hypothalamus in vitro. Brain Res. 326: 41-46.
8. Osborne, H., Przewlocki, R., Hollt, V. and Herz, A. (1979). Release of β-endorphin from rat hypothalamus in vitro. Eur. J. Pharmacol. 55: 425-428.
9. Fukata, J., Nakai, Y. and Imura, H. (1980). Release of immunoreactive β-endorphin from rat hypothalamic fragments in vitro. Brain Res. 201: 492-496.
10. Nikolarakis, K. E., Almedia, O. F. X. and Herz, A. (1986). Stimulation of hypothalamic β-endorphin and dynorphin release by corticotropin-releasing factor (CRF). Brain Res. 399: 152-155.
11. Rasmussen, D. D., Liu, J. H., Wolf, P. L. and Yen, S. S. C. (1987). Neurosecretion of human hypothalamic immunoreactive β-endorphin: In vitro regulation by dopamine. Neuroendocrinology 45: 197-200.
12. Akil, H., Young, E., Watson, S. J. and Coy, D. H. (1981). Opiate binding properties of naturally occurring N- and C-terminus modified beta-endorphin. Peptides 2: 289-292.
13. Deakin, J. F., Dostrovsky, J. O. and Smyth, D. G. (1980). Influence of N-terminal acetylation and C-terminal proteolysis on the analgesic activity of beta-endorphin. Biochem. J. 189: 501-506.
14. Bals-Kubik, R., Herz, A. and Shippenberg, T. S. (1988). β-endorphin-(1-27) is a naturally occurring antagonist of the reinforcing effects of opioids. N.-S. Arch. Pharmacol. 338: 392-396.
15. Nicolas, P., Hammonds, R. G., Jr. and Li, C. H. (1984). Beta-endorphin analgesia is inhibited by synthetic analogs of beta-endorphin. Proc. Natl. Acad. Sci., USA 81: 3074-3077.
16. Young, E. A., Lewis, J. and Akil, A. (1986). The preferential release of beta-endorphin from the anterior pituitary lobe by corticotropin releasing factor (CRF). Peptides 7: 603-607.
17. Akil, H., Shiomi, H. and Matthews, S. (1985). Induction of the intermediate pituitary by stress: Synthesis and release of a non-opioid form of beta-endorphin. Science 277: 424-426.
18. Ham, J. and Smyth, D. G. (1986). Chronic stimulation of anterior pituitary cell cultures with CRF leads to the secretion of lipotropin. Neuroendocrinology 44: 433-438.
19. Loh, Y. P. and Jenks, B. G. (1981). Evidence for two different turnover pools of adrenocorticotropin, alpha melanocyte stimulating hormone, and endorphin-related peptides released by the frog pituitary neurointermediate lobe. Endocrinology 109: 54-61.
20. DeSouza, E. B., Insel, T. R., Perrin, M. H., Rivier, J., Vale, W. W. and Kuhar, M. J. (1985). Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: An autoradiographic study. J. Neurosci. 5: 3189-3203.
21. Tribollet, E., Barberis, C., Jard, S., Dubois-Dauphin, M. and Dreifuss, J. J. (1988). Localization and pharmacological characterization of high affinity binding sites for vasopressin and oxytocin in the rat brain by light microscopic autoradiography. Brain Res. 442: 105-118.
22. Biegon, A., Rainbow, T. C. and McEwen, B. S. (1982).

Quantitative autoradiography of serotonin receptors in the rat brain. Brain Res. 242: 197-204.

23. Chen, C. L., Dionne, F. T. and Roberts, J. L. (1984). Regulation of pro-opiomelanocortin mRNA levels in rat pituitary by dopaminergic compounds. Proc. Natl. Acad. Sci., USA 80: 2211-2214.

24. Meador-Woodruff, J. H., Pellerito, B., Bronstein, D., Lin, H.-L. and Akil, H. Differential effects of haloperidol on the rat pituitary: Decreased biosynthesis, processing and release of anterior lobe proopiomelanocortin. Neuroendocrinology (in press).

25. Vale, W. W., Rivier, C., Spiess, J. and Rivier, J. (1983). Corticotropin-releasing factor. In: Krieger, D. T., Martin, J. B. and Brownstein, M. J. (eds) Brain Peptides, Wiley, New York p. 961-974.

26. Vermes, I., Mulder, G. H., Smelik, P. G. and Tilders, F. J. H. (1980). Differential control of β-endorphin/β-lipotropin secretion from anterior and intermediate lobes of the rat pituitary gland in vitro. Life Sci. 27: 1761-1768.

27. Spinedi, E. and Negro-Vilar, A. (1983). Serotonin and adrenocorticotropin (ACTH) release: direct effects at the anterior pituitary level and potentiation of arginine vasopressin-induced ACTH release. Endocrinology 112: 1217-1223.

28. Rivier, C. and Vale, W. (1983). Interaction of corticotropin-releasing factor and arginine vasopressin on adrenocorticotropin secretion in vivo. Endocrinology 113: 939-942.

29. Gillies, G. E., Linton, E. A. and Lowry, P. J. (1982). Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. Nature 299: 355-357.

30. Fuxe, K. and Jonnson, G. (1974). Further mapping of central 5-hydroxytryptamine neurons: studies with the neurotoxic di-hydroxytryptamines. Adv. Biochem,. Psychopharmacol. 10: 1-12.

31. Sawchenko, P. E., Swanson, L. W. and Vale, W. (1984). Corticotropin-releasing factor: co-expression within distinct subsets of oxytocin-, vasopressin-, and neurotensin-immunoreactive neurons in the hypothalamus of the male rat. J. Neurosci. 4: 1118-1129.

32. Akil, H., Ueda, J.-L., Lewis, J. W., Walker, J. M., Shiomi, H., Liebeskind, J. C. and Watson, S. J. (1981). Multiple forms of beta-endorphin (βE) in pituitary and brain: Effect of stress. In: Takagi, H. and Simon, E. (eds) Advances in Endogenous and Exogenous Opioids, Kodansha Press, Tokyo, p. 116-118.

33. Millan, M. J., Przewlocki, R., Jerlicz, M., Gramsch, Ch., Hollt, V. and Herz, A. (1981). Stress-induced release of brain and pituitary β-endorphin: major role of endorphins in generation of hyperthermia, not analgesia. Brain Res. 208: 325-338.

34. Shiomi, H., Watson, S. J., Kelsey, J. E. and Akil, H. (1986). Pretranslational and posttranslational mechanisms for regulating β-endorphin-adrenocorticotropin of the anterior pituitary lobe. Endocrinology 119: 1793-1799.