paraventricular, supraoptic and aarcuate nucleus. The distribution pattern of these three neurohormone immunoreactive cells, however, were apparently different. The MET-ENK immunoreactive cell group were also found in the ventromedial nucleus and NT positive cells were found in the lateral hypothalamus. A small number of β-END immunoreactive nerve terminals were found in the internal layer of the median eminence. Two weeks after hypophysectomy, a number of β-END immunoreactive nerve terminals appeared in the external and internal layer of the median eminence. In the median eminence of the normal control rat, a dense meshwork of the MET-ENK and NT immunoreactive nerve terminals was observed exclusively in the external layer abbuting on the portal vessels. The elution-restaining technique did not show any co-existence of these three neurohormones in the same neuron of the hypothalamus, although the above-mentioned two or three neurohormones have been reported to co-exist in various combination in the same cell of the anterior or medial lobe of the hypophysis.

The present data suggest that β-END, MET-ENK and NT have distinct neuronal system in the hypothalamus and that β-END as well as MET-ENK and NT is released into the portal vessels of the median eminence to modulate the anterior pituitary function.

10. Involvement of Calcium Ion in the Release of Immunoreactive β-endorphin-like Peptide from Dispersed Cells of the Neurointermediate Lobe of the Rat Pituitary Gland

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Ca^{2+} plays an important role in hormone secretion. It was reported that Ca^{2+} is essential for the release mechanism of pituitary hormones. We investigated the involvement of Ca^{2+} and adenosine 3′,5′-monophosphate (cAMP) system in the release of immunoreactive β-endorphin-like peptide (IR-β-EP). The intermediate lobe of the rat pituitary gland is a homogeneous population of cells.

cAMP analogs, phosphodiesterase inhibitor, L-isoproterenol, cholera toxin and forskolin stimulated the release of IR-β-EP from the dispersed cells of the neurointermediate lobe rat pituitary gland. A calcium antagonist (verapamil) inhibited the effects of these stimulants, but cAMP accumulation was not inhibited. High K^+ stimulated the release of IR-β-EP without cAMP accumulation. Na^+ ionophore, monensin enhanced the release of IR-β-EP, but did not stimulate the formation of cAMP.

These findings suggest that Ca^{2+} and cAMP system might be participated in the release mechanism of IR-β-EP. Intra-and extracellular Ca^{2+} may have an essential role in the release of pituitary hormone secretion.

11. β-endorphin and Gonadotropin in Estradiol Induced Feedback Test —Comparison between Normal Cycle and Hypothalamic Amenorrhea—

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Plasma variations of β-endorphin (β-E) and gonadotropin (Gn) in estradiol induced positive feedback test were investigated in 16 normal women and 8 hypothalamic amenorreheic women (HA). After intramuscular injection of estradiol benzoate (EB) 1 mg, serial determinations of plasma immunoreactive β-E (ir-β-E), ACTH, LH, FSH, estradiol were made at 24 hours interval. Ir-β-E was extracted using ODS-silica and was measured by RIA which crossreacts 100% with β-lipotropin.

Although the basal ir-β-E levels of both groups were not significantly different, the pattern of response to EB was different. In normal women, plasma ir-β-E increased to 140.7% (against basal level, mean) at 48 h when Gn showed small decrement and decreased to 85.1% at 72 h when Gn showed positive surge (LH: 365.4%, FSH: 165.3%). While in patients with HA, both ir-β-E and Gn revealed no marked response. ACTH did not show any significant change.

Decreased responsiveness of Gn and β-E to estradiol in HA suggests the impaired release mechanism of GnRH from hypothalamus as well as that of β-E. In normal menstrual cycle, inverse relationship between Gn and β-E suggests that Gn release might be under tonic influence by β-E mechanism.

12. Low Dose Intermittent Infusion of Naloxone to Variable Gonadal Conditions