EPITHEORESE KLINIKES FARMAKOLOFIAS
KAI FARMAKOKINETIKES, INTERNATIONAL
EDITION 13: 86(1999)

Corticotropin-Releasing Hormone Binding Protein (CRH-BP) is Expressed in Normal and Tumoral Cells of Rat Adrenal Medulla

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Corticotropin releasing hormone (CRH), a hypothalamic neuropeptide, is the principal neuroregulator of the hypothalamus-pituitary adrenal axis. CRH is also produced by a number of peripheral tissues, including adrenals (1). We have recently reported that CRH is expressed by both normal rat adrenal chromaffin cells and the widely used PC12 rat pheochromocytoma cell line (2). It seems that the capacity to produce CRH is a property of normal adrenal chromaffin cells and that the production of CRH by the PC12 cells may represent the preservation of a normal chromaffin cell characteristic rather than a tumor-induced ectopic phenomenon.

The physiological role of adrenal CRH is slowly unfolding. All available evidence indicates that CRH plays a significant role in the adrenal medulla, acting as a paracrine modulator of adrenal physiology. Recent data support this hypothesis, demonstrating a dose-depended stimulatory effect of CRH on the production of catecholamines from both normal and PC12 tumoral rat adrenal chromaffin cells (2). This hypothesis is further supported by findings showing the presence in this tissue of functional CRH receptors (3). Assessment of the functional role of adrenal CRH requires characterization of the CRH system, which includes ligand, receptor, and the high affinity CRH-binding protein. Recently, a 37-kDa high affinity CRH-binding protein (CRH-BP) was purified and cloned from human plasma (4,5). It was shown that CRH-BP is capable of competing with ligand for binding to the CRH receptor and thus may function to modulate CRH action. In addition to its presence in human plasma, CRH-BP was found in brain, pituitary, liver, and placenta (6). Accordingly, in the present study we have evaluated the gene expression and gene product of the CRF-BP in normal rat adrenal medulla cells as well as in the PC12 rat pheochromocytoma cell line.

To evaluate the presence of CRH-BP transcript in rat adrenals, we performed Reverse-Transcription Polymerase Chain Reaction (RT-PCR) experiments. RT-PCR was used to amplify the mRNA signal of CRH-BP in RNA from normal rat adrenal medulla cells as well as in PC12 rat pheochromocytoma cells. RNA from rat brain areas which express the CRH-BP gene (hypothalamus, cortex, amygdala) were used as positive controls (6). Using specific primers for rat CRH-BP, amplified products were evident in RNA preparations from all positive control tissues as well as from both normal adrenal medulla and PC12 cells. When the RT-PCR proceeded in the absence of reverse transcriptase, they were no

amplification products in both adrenal and brain cells. The presence of the translation product of the CRH-BP mRNA was confirmed by Western blot analysis, using a specific antibody for rat CRH-BP, kindly provided by Dr W. Vale (Salk Institute, La Jolla, CA). A 37-kDa protein was detected in extracts from all positive brain tissues and from both normal adrenal medulla and PC12 cells. Immunohistrochemistry analysis of normal rat adrenals showed the presence of immunoreactive CRH-BP in chromaffin cells of the medulla. A weak but significant staining was also observed in the cortical cells. The presence of immunoreactive CRH-BP was also confirmed in the PC12 rat pheochromocytoma cells, using immunofluoresence microscopy. Specific staining was found in both the cytoplasm and the membrane of these cells.

In conclusion, our data provide strong evidence for the expression of CRH-BP in both normal and tumoral cells of rat adrenals. The presence in this tissue of all components of the CRH system, i.e CRH, CRH receptors and CRH-BP points out to a significant role of CRH in the adrenal physiology. It is possible that adrenal CRH-BP regulates the bioavailability of locally-produced CRH, modulating its paracrine actions.