Hormones and their Actions
Part I

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CHAPTER 9

Internalization of peptide hormones and hormone receptors

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1. Introduction

Peptide hormones are one class of many agents present in the bloodstream that affect the multiplication and differentiated functions of mammalian cells. The ability of a particular peptide hormone to elicit an effect in the appropriate target cell is dictated by the presence of receptors on the surface of the target cell which specifically bind that hormone. Although the cellular responses to the different peptide hormones vary, as do many of the mechanisms of signal transduction that translate the binding of the hormone to the cellular response, there is one salient feature that all peptide hormones studied to date share. This is the receptor-mediated endocytosis (RME) of the hormone.

The idea that proteins could be internalized by a receptor-mediated mechanism by their target cells was sparked by the pioneering studies of Goldstein and co-workers [1] and by Cohen and co-workers [2,3], who obtained evidence for the receptor-mediated internalization and degradation of low-density lipoprotein (LDL) and epidermal growth factor (EGF), respectively, in the mid 1970s. Although endocytosis of a non-specific nature had been described by then, the concept of endocytosis of a specific ligand being mediated by the binding of that ligand to a cell surface receptor was unprecedented.

These investigators were one of the first to study the binding of ^{125}I-labelled ligands to intact cells (as opposed to studying the binding of the ligand to membranes, which was the prevailing approach at the time). Interestingly, their studies showed that when the binding studies on the cultured cells were performed at 37°C, but not at 4°C, there was a time-dependent accumulation of degradation products

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Abbreviations and trivial names used are: RME, receptor-mediated endocytosis; LDL, low density lipoprotein; EGF, epidermal growth factor; SDS, sodium dodecyl sulfate; LH, luteinizing hormone; hCG, human chorionic gonadotropin; and G protein, guanine nucleotide binding protein.

of the ligand in the culture medium. That the degradation of these ligands was occurring as a result of internalization of the ligand into the cell was suggested by observations that the accumulation of degradation products in the medium was both energy- and temperature-dependent and that it could be inhibited by agents known to inhibit lysosomal function. By using specific treatments to release the surface-bound [^{125}I]LDL or [^{125}I]EGF, it was possible to document the appearance of intracellular radioactivity (representing intact or partially degraded ligand) prior to the release of degradation products into the medium. Furthermore, it was found that some compounds (such as metabolic inhibitors) prevented the accumulation of intracellular ligand (presumably by inhibiting internalization); whereas other compounds known to inhibit lysosomal function (such as NH_4Cl or chloroquine) allowed internalization, but prevented degradation of the ligand [3–7].

Concomitant morphological studies by electron microscopy on the fates of receptor-bound LDL and EGF (using ligands covalently attached to electron-dense ferritin) elegantly confirmed the inferences from the biochemical data that these ligands were internalized and degraded in the lysosomes [8–11]. Since the internalization and degradation of ligand was strictly dependent upon binding of the ligand to the cell surface receptor, this process was called receptor-mediated endocytosis (RME).

RME has since been shown to occur with other transport proteins, other growth factors, and with peptide hormones (for reviews see Refs. 12–16). The general features of RME as they are understood today from biochemical and morphological studies on a variety of ligands are discussed below as they pertain to peptide hormones.

2. *General features of receptor-mediated endocytosis*

A schematic overview of RME is shown in Fig. 1. The cell surface receptors for a particular hormone are either located in areas of the plasma membrane referred to as coated pits or they are randomly distributed throughout the cell surface and migrate to the coated pits upon binding of the hormone. Coated pits are indented areas of the plasma membrane where there is an intracellular 'lining' of the membrane with the protein clathrin and they constitute a small percentage (<5%) of the total area of the plasma membrane [8,17,18]. In the cases where the hormone-receptor complexes migrate to coated pits, there often is a microaggregation of the complexes (two to four per group) during this redistribution [19]. Following this microaggregation there is a more masssive clustering of hormone-receptor complexes in the coated pits.

Coated pits containing receptor-bound hormones become invaginated and pinch off intracellularly to form what are called coated vesicles. The coated vesicles still have clathrin associated with them, forming basket-like structures around the ves-

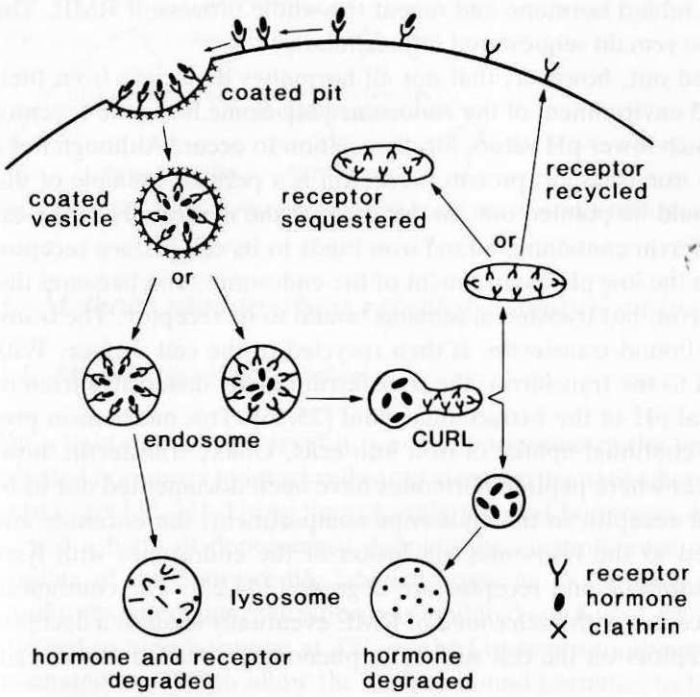

Fig. 1. Schematic representation of the possible routes of receptor and hormone during RME.

icles [20]. The lumen (fluid-filled interior) of the coated vesicles does not have any free hormone. At this stage, the hormone is still bound to the receptor, facing the lumen [10]. With time, the coated vesicles shed their clathrin coats and fuse with other similar vesicles; all this time these vesicles are moving further into the interior of the cell [15]. The prelysosomal vesicles resulting from these fusions are called endosomes or endocytic vesicles and have a critical role in RME due to the acidic environment of their lumen.

Although not as acidic as lysosomes (with an intra-compartmental pH of 4.5, see Ref. 21), the pH 5.5 environment of the endosome [22] is sufficiently low to cause the dissociation of some hormones from their receptors. When this occurs, there is a subsequent sequestering of the free hormone from the receptor in a related vesicle and tubule compartment called CURL (compartment for uncoupling of receptor from ligand, see Ref. 23), where the free hormone is sequestered into the vesicular structure while the receptor accumulates in the membrane of the tubule structure. A subsequent physical separation of these compartments allows for the differential processing of the hormone versus the receptor. Thus, while the free hormone is ultimately delivered (via vesicle fusion) to the lysosome where it is degraded, the free receptor may be recycled (via the Golgi compartment) to the cell

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