Abstract

The cellular distribution of unliganded and liganded glucocorticoid hormone receptor (GR) has been studied for many years but remains controversial. The consensus opinion in the field is probably still the two-step mechanism of activation, involving a hormone-induced translocation of GR from the cytoplasm to the cell nucleus. The evidence for such a process is, however, ambiguous.
The localization of heat shock protein Mr 90,000 (hsp90) is also somewhat controversial regarding possible nuclear distribution and the effect of heat stress.
We have studied the immunocytological distribution of GR and hsp90 in a number of mammalian cell types with emphasis on human fibroblasts. The analyses have included a variety of culture conditions, treatment with glucocorticoid hormones, heat stress and metabolic inhibitors. We have analyzed the effect of several different fixation/permeabilization techniques and anti-GR antibodies and have used several mono- and double-staining techniques including confocal laser scanning microscopy, computerized image analysis and photometry of GR- and hsp90- intensities on thin optical sections of nuclei and cytoplasm. The results have been analyzed statistically.

Endogenous GR and hsp90 were detected in both the nucleus and cytoplasm in all analyzed cell types. Various fixations/permeabilizations extracted different amounts of GR from cells without affecting the relative nuclear to cytoplasmic distribution. Nuclear GR was diffuse in all and, in addition, granular in certain cells, whereas hsp90 was always diffusely distributed. Cytoplasmic GR was either fibrillar or non-fibrillar depending on the cell type. Fibrillar GR colocalized with cytoplasmic microtubules (MTs). Cytoplasmic hsp90 was essentially diffuse. During cell division, GR was localized in the mitotic spindle apparatus. Hsp90 was sometimes observed in the metaphase spindle, but was otherwise not present in the mitotic structures.
GR colocalized with the MT-protein tubulin during the spontaneous redistribution of MTs during the cell cycle and also after drug-induced depolymerization of MTs. Furthermore, tubulin co-purified with GR and GR co-polymerized with newly formed MTs *in vitro*.
Taken together, these observations indicate that GR is a microtubule associated protein, MAP.
Glucocorticoid hormones did not change the cellular distribution of either GR or hsp90. However, precipitating fixation revealed a reduced extraction of activated GR. This was interpreted as reflecting that glucocorticoid hormone treatment induces an increase in GR-affinity for its docking sites in both nucleus and cytoplasm.
Heat-stress induced a true nuclear translocation of hsp90, but not of GR, independent of RNA- or protein- synthesis or of an intact cytoskeleton, however, only detectable after precipitating fixation. The translocation was rapidly reversible upon elimination of the heat stressor.
Glucocorticoids inhibited MT-assembly in an *in vitro* MT-polymerization assay. In the same assay, purified hsp90 tended to increase MT-assembly, whereas purified GR was inactive.

The observed interaction between GR and tubulin represents evidence of GR being associated with the cytoskeleton. Future work will be directed towards understanding the putative physiological relevance of this interaction.

Key words: Glucocorticoid receptor (GR), glucocorticoid hormone, heat shock protein (hsp), cytoskeleton, microtubule (MT), tubulin, microtubule associated protein (MAP), mitotic spindle, subcellular localization, nuclear translocation, mechanism of hormone action, fibroblast, immunocytology, confocal laser scanning microscopy (CLSM), morphometry, heterogeneity.

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