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Effects of GnRH agonist treatment on GnRH receptors in human prostate cancer cells: an atomic force microscopy study

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We previously demonstrated by Western blotting and immunocytochemistry that a GnRH agonist (leuprorelin acetate, LA) was able to induce a post-transcriptional increase in GnRH receptor (GnRH-R) expression at the plasma membrane of androgen-sensitive (LNCaP) and -insensitive (PC-3) prostate cancer (PCa) cells. In the present study, we used atomic force microscopy (AFM) to gain a deeper insight into the effects of LA on the behaviour of GnRH-R in highly invasive and poorly differentiated PC-3 cells. The use of this powerful, non-destructive technique allows to identify and study the biological features of the living cell surface, as ligand-receptor interactions. Here, we investigated for 6, 12, 18, 24 and 30 days, the effect of LA (10-11 and 10-6 M) in PC-3 cells on: i) amount of LA/GnRH-R binding events (i.e. GnRH-R quantification), ii) strength of the analogue-receptor binding, iii) receptor topography. Briefly, analogue molecules were immobilized onto conical AFM tips and the single agonist/receptor interactions were measured by force-distance cycles.

In agreement with our previous results, the number of GnRH-R augmented during 30 days due to the effect of LA treatment. The increasing rate of GnRH-R was dose-dependent until the 24th day and reached the maximum (~70%) after 30 days of treatment with the highest dose of LA (10-6M).

At least 2 different receptor bound strengths have been detected, probably due to the presence of two GnRH-R classes. The majority of the sites showed a relatively low bound strength (~37 piconewton).

A LA/GnRH-R complex lifetime of ~ 9s and ~3.4 s for the higher and lower bound strength receptors, respectively, has been determined.

Regarding GnRH-R topography, a homogeneous distribution of the binding events has been found on untreated and LA-treated PC-3 cell surfaces.

The persistence of high receptor levels at the androgen-insensitive cell surface may warrant the maintenance of the response to the analogue in androgen-unresponsive PCa also, which might be useful in clinical practice. Moreover, the definition of parameters as ligand/receptor bond strength and lifetime could shed light on the poorly understood molecular basis of LA/GnRH-R interaction and might be used to address structural/chemical agonist optimizations.

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