

**P016** Inverse agonist exposure enhances ligand binding and G protein activation of the human MT1 melatonin receptor, but leads to receptor down-regulation

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The pineal hormone melatonin, secreted during the hours of darkness, binds and activates G protein-coupled melatonin receptors. The density and affinity of the endogenous melatonin receptors change throughout the 24-hour day, and the exposure of recombinant melatonin receptors to melatonin results in desensitization of the receptors. Receptor density, G protein activation, and expression level were analyzed in CHO cell lines stably expressing the human MT1 receptors after 1 or 72 h exposure to melatonin (agonist, 10 nM) and luzindole (antagonist / inverse agonist, 10  $\mu$ M). The 72 h exposure to luzindole significantly increased the apparent receptor density in cell lines with both high and low MT1 receptor expression levels (MT1<sub>high</sub> and MT1<sub>low</sub> cells, respectively). In the constitutively active MT1<sub>high</sub> cells, luzindole pre-treatment also stimulated the functional response to melatonin in [<sup>35</sup>S]GTP $\gamma$ S binding assays at both time points, whereas melatonin pre-treatment attenuated functional responses. In MT1<sub>high</sub> cells, luzindole pre-treatment decreased the total cellular level of MT1 receptor protein at both time points, but increased the plasma membrane expression of the receptor at 72 h. Melatonin significantly internalized MT1 receptors in MT1<sub>high</sub> cells after a 1 h treatment. These results indicate that agonist treatment desensitizes MT1 receptors, whereas the inverse agonist luzindole increases ligand binding and G protein activation. Luzindole also stimulates down-regulation of the MT1 receptor protein, interfering with the synthesis and / or degradation of the receptor.