144P

Agonist induced desensitization of the human beta$_3$-adrenoceptor

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Beta$_3$-Adrenoceptors mediate the relaxation of smooth muscle in e.g. the urinary bladder, the gastrointestinal tract and several blood vessels. Beta$_3$-Adrenoceptor agonists are currently in clinical trials for the treatment of voiding disorders and depression, both requiring chronic treatment. Early studies on beta$_3$-adrenoceptors expressed in Chinese hamster ovary (CHO) cells indicated that the beta$_3$-adrenoceptor is rather resistant towards agonist-induced desensitization, possibly due to a lack of phosphorylation sites; on the other hand, desensitization may occur in human embryo kidney (HEK293) cells (Chaudhry *et al.* 1994). The underlying mechanisms are largely unknown. Therefore, we have compared desensitization in CHO and HEK293 cells transfected with human beta$_3$-adrenoceptors and investigated underlying mechanisms in the latter.

CHO and HEK293 cells were stably transfected with the human beta$_3$-adrenoceptor at a density of approximately 200 and 100 fmol/mg protein, respectively. Unless otherwise indicated cells were treated for 24 hours with 10 μM isoprenaline or vehicle. After washing 3 times with buffer, agonist was added for 30 minutes in the presence of 100 μM each of the phosphodiesterase inhibitors isobutylmethyl-xanthine and Ro20-1724 (4-[(3-butoxy-4-methoxyphenyl)-methyl]-2-imidazolidinone) using a LANCE® kit (Perkin Elmer) as described (Jongsma *et al.,* 2006). Data are means ± SEM of 3-6 experiments, and a $p<0.05$ (t-test) was considered significant.

Isoprenaline pre-treatment had little effect on the subsequent on cAMP responses to isoprenaline in CHO cells, but reduced the maximal response in HEK293 cells from 712 ± 33 to 260 ± 16 ($p<0.05$) without major chances of agonist potency, and all further experiments were done in HEK293 cells. The desensiti-zation of the cAMP response was time and concentration dependent. Treatment with pertussis toxin (100 ng ml^{-1} for 24 h) did not alter the desensitization. Moreover isoprenaline pre-treatment also reduced the forskolin induced cAMP accumulation to an at least similar extent. A 24 h treatment with 10 μM forsko-lin for 24 hours also desensitized the subsequent response to isoprenaline. The common beta$_3$-adreno-ceptor polymorphism Trp64Arg did not affect its susceptibility towards agonist-induced desensitization.

We conclude that the agonist induced desensitization of the human beta$_3$-adrenoceptor is cell-type specific. It does functionally not involve G_i protein, but may involve reduced adenylyl cyclase function, possibly explaining why it can happen despite a lack of phosphorylation sites. The desensitization can be mimicked by adenylyl cyclase activation and is not affected by the Trp64Arg polymorphism.

Chaudhry *et al*, Influence of cell type upon the desensitization of the beta 3-adrenergic receptor. J. Pharmacol. Exp. Ther., 1994, 271, 1253-8
Jongsma *et al*, BML-241 fails to display selective antagonism at the sphingosine-1-phosphate receptor, S1P(3), Br. J. Pharmacol., 2006, 149, 277-82