Naunyn-Schmiedeberg's Arch Pharmacol (2013) 386:841–842
DOI 10.1007/s00210-013-0884-x

EDITORIAL

A door opener for future research: agonist-induced β_3-adrenoceptor desensitization in HEK cells but not CHO cells

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Received: 11 May 2013 / Accepted: 21 May 2013 / Published online: 12 June 2013

Overactive bladder syndrome (OAB) is an important medical condition. So far, treatment options are limited. Recently, the β_3-adrenoceptor (β_3AR) agonist mirabegron has been introduced into the clinic for treatment of OAB (Sacco and Bientinesi 2012; Igawa and Michel 2013). Binding of an agonist to the β_3AR induces activation of the G protein G_s with subsequent stimulation of adenylyl cyclase (AC)-mediated cAMP formation (Emorine et al. 1991). cAMP then induces relaxation of the detrusor muscle of the bladder and, thereby, ameliorates the clinical symptoms of OAB. Chronic exposure of G protein-coupled receptors (GPCR) to agonist leads to desensitization, i.e., reduction of agonist responses and clinical efficacy of the drug (Gainetdinov et al. 2004). GPCR desensitization is relevant for various medical conditions including neuropsychiatric disorders and treatment of bronchial asthma with β_2-adrenoceptor agonists (Gainetdinov et al. 2004; Mochizuki et al. 2012). Since OAB is a chronic medical condition, desensitization is a relevant concern for this condition, too. However, the question whether the β_3AR is subject to desensitization is controversial (Nantel et al. 1993).

In this issue of *Naunyn-Schmiedebergs Archives of Pharmacology*, Michel-Reher and Michel (2013) expressed the β_3AR in human embryonic kidney (HEK293) cells and Chinese hamster ovary (CHO) cells. Strikingly, the β_3AR expressed in HEK293 cells but not the β_3AR expressed in CHO cells undergoes agonist-induced desensitization. Desensitization is dependent on the agonist concentration and agonist exposure time, cAMP accumulation being the read-out parameter. Apparently, G_i proteins are not involved in the process because the ADP-ribosylating bacterial toxin pertussis toxin does not affect the process. Additionally, desensitization has no effect of β_3AR expression density and affinity, arguing against the receptor itself being the target of desensitization. However, the direct AC activator forskolin effectively reduces β_3AR-mediated cAMP accumulation, and conversely, β_3AR-agonist stimulation effectively reduces forskolin-mediated cAMP accumulation. These data strongly suggest that ACs are the target of β_3AR-mediated desensitization.

This paper raises several questions. It is puzzling why β_3AR desensitization is so cell type-specific. As a first step, the expression pattern of AC isoforms in HEK- and CHO cells has to be studied. Such studies are straightforward at the mRNA level (Kinast et al. 2012) but highly problematic at the protein level because of the questionable quality of AC antibodies (Göttle et al. 2009). Should there be an AC isoform that is expressed exclusively in HEK cells but not in CHO cells, then the corresponding AC isoform could be knocked down with the siRNA technique. Conversely, expression of a defined AC isoform in CHO cells may reconstitute desensitization of the β_3AR in these cells. Unfortunately, there is a paucity of isoform-specific and cell membrane-permeable AC inhibitors that could complement such genetically oriented studies (Seifert et al. 2012).

Another question is how β_3AR agonists and forskolin induce desensitization. Formally, it is possible that the effect of β_3AR agonists is mediated via cAMP-dependent or cAMP-independent mechanisms. Accordingly, the effects of other cAMP-elevating compounds such as phosphodiesterase inhibitors and membrane-permeable cAMP analogs should be studied. Moreover, the effects of inhibitors of cAMP-dependent protein kinase (PKA) should be examined. In fact, it has already been shown that at least in

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adipocytes, the β_3AR can activate both PKA-dependent and PKA-independent signal transduction pathways (Tchivileva et al. 2009). While the involvement of G_i mediated signaling seems unlikely in the desensitization, non-canonical G_q- and/or β-arrestin-mediated signal transduction should be considered too (Reiter et al. 2012).

Conversely, the precise mechanism by which forskolin induces desensitization of the β_3AR is still unknown. While forskolin is an activator of AC isoforms 1–8 (Seifert et al. 2012), the diterpene can also exert off-target effects on other proteins (Laurenza et al. 1989). An approach to discriminate between AC-dependent and AC-independent effects of forskolin is to conduct structure–activity relationship studies for diterpenes, 1-deoxy forskolin analogs being without stimulatory effect on ACs (Laurenza et al. 1989; Seifert et al. 2012). Along the same line, the effects of PKA inhibitors on forskolin-induced desensitization have to be studied. Furthermore, it remains to be determined whether desensitization takes place directly at the level of AC or indirectly at a protein interacting with AC (Ostrom et al. 2012; Scott et al. 2013). Most importantly, once the molecular mechanisms of β_3AR desensitization have been worked out in mammalian expression systems, this process should also be studied in human detrusor muscle samples in order to determine the clinical relevance of the intriguing observation in the paper by Michel-Reher and Michel.

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