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Orexin Receptors and G-Protein Coupling: Evidence for Another “Promiscuous” Seven Transmembrane Domain Receptor

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Following the meritorious work by Sakurai et al. in 1998 (1), on the discovery of orexins and their receptors, numerous studies have confirmed the importance of these peptides and their receptors not only in the central nervous system, but also in peripheral organs. Although expression studies of these peptides and their receptors are well underway in human and animal models, little is known about their detailed signaling characteristics. A recent paper by Zhu et al. (2) provides an insight into the individual signaling of orexin 1 (OX1R) and orexin 2 (OX2R) receptors.

In their study, Zhu et al. have shown that OX1R couples exclusively to pertussis toxin (PTX)-insensitive G proteins, while OX2R couples to both PTX-sensitive and -insensitive G proteins. In forskolin-stimulated cells expressing either OX1R or OX2R, only the latter appeared to inhibit cAMP production by orexin-A (OR-A), an effect abolished by pretreatment with PTX. Furthermore the authors demonstrated that treatment with OR-A and OR-B induced an increase in intracellular Ca^{2+} in OX1R- or OX2R-expressing cells. These responses were not affected by PTX pretreatment. In a neuronal cell line that expresses endogenously the OX2R, a phospholipase C (PLC)-inhibitor inhibited orexin-mediated neuronal activation. These data confirm previous findings from our laboratory where we have shown that OX2R in human tissue can couple to both PTX-sensitive and -insensitive G-protein α -subunits (3).

In the study by Zhu et al., although an effect of OR-A on forskolin-induced cAMP is shown, there is no data regarding the regulation of cAMP by orexins, although a few studies have suggested a link between the two. In rat adrenocortical cells, for example, orexins stimulate corticosterone secretion through the activation of the

adenylate cyclase-dependent signaling cascade (4); similarly, in human adrenocortical cells, OR-A stimulates cortisol secretion through activation of cAMP (5). Furthermore, previous studies from our group have elucidated that the human adult adrenal OX2R can couple to the α -subunits of Gs, Gq, and to a lesser effect Gi, activating both adenylate cyclase and PLC pathways (6). Indeed, we have mapped out that there is selective and differential activation of PTX-sensitive G proteins, with no coupling to Go (3, 6).

These fundamental differences in G-protein coupling can be influenced by a number of factors. Receptor density of G-protein-coupled receptors appears to influence differences in coupling and signaling. When 5-hydroxytryptamine 5-HT₂ receptors are expressed at low density, they couple to the Gq/PLC pathway, maintaining their ability to enhance phosphoinositol hydrolysis, whereas a more modest cAMP production was noted (7). Therefore, it appears that the ability of orexin receptors to interact with different G proteins, apart from being receptor-subtype specific, can also be influenced by receptor density.

In addition, the authors should have emphasised the fact that this is an *in vitro* study, where tissue specificity is not taken under consideration. For example, the induction of cAMP in the adrenal cortex might be a tissue-dependent phenomenon, since in PC12 cells, OR-A and -B inhibited the PACAP-induced increase in the cAMP (8). Other events, such as development and changes in the energy status, for example, might influence orexin receptor expression and signaling characteristics. As we have mentioned previously, in the human adrenal cortex, there is a “switch” of G-protein coupling of orexin receptors; in the human fetal adrenal cortex, OX2R couples to Gs and Gi, whereas in the adult adrenal, there is a change towards Gq and less towards Gi (3, 6). We

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have also demonstrated that energy status might also exert potent effects on altering the G-protein coupling in the rat adrenal cortex (9). In the adrenal cortex of the food-deprived animal, compared to animals fed ad libitum, there was decreased coupling of orexin receptors to Gs and Go and increased coupling to Gi, yet there was no coupling towards Gq (9). Similarly, food deprivation led to differential G-protein activation in the rat hypothalamus (9). Hence, with the widespread expression of orexin receptors throughout the body, it is attractive to speculate that orexin receptors couple to G proteins in such a way, in order to regulate energy demands and tissue-specific functions (Fig. 1).

In addition to these observations, an interesting study by Hilaiet et al. has shown that when the cannabinoid receptor CB1 and OX1R are co-expressed, there is a major CB1-dependent enhancement of the OR-A potency to activate the mitogen-activated protein kinase pathway (10). In the same study, using electron microscopy, it was shown that CB1 and OX1R are closely apposed at the plasma membrane level to form heterodimers. In another study, Bioluminescence Resonance Energy Transfer (BRET) analysis showed that OX1R is

capable of forming a heterodimer with OX2R (11).

In view of all these exciting findings, it is becoming apparent that orexin receptors are highly “promiscuous” not only towards different G-protein α -subunits, but also towards other G-protein-coupled receptors. These novel interactions open a new chapter in the regulation and signaling characteristics of orexin receptors. Any future attempts to elucidate signaling characteristics in systems where they express orexin receptors -in vivo or in vitro- should be done taking under consideration these interactions.

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Fig. 1. Diagrammatic representation of altered G-protein coupling of the human adrenal orexin receptors (panel A) and the rat adrenal orexin receptors (panel B) upon changes in development and energy status, respectively.

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