

β_3 -Adrenoceptors: a drug target in ophthalmology?

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In this issue of the journal, Dal Monte and colleagues report on the role of β_3 -adrenoceptors in the regulation of angiogenesis responses to hypoxia in the retina (Dal Monte et al. 2013). After original ideas of using β_3 -adrenoceptor agonists in the treatment of obesity and type 2 diabetes mellitus have been disproven (Michel et al. 2011), current thinking on this drug class is largely focused on the treatment of overactive urinary bladder, and the first two compounds have reported to have clinical efficacy in this indication (Khullar et al. 2013; Ohlstein et al. 2012). Against this background, we will shortly summarize the emerging evidence for a role of β_3 -adrenoceptors as a therapeutic target in ophthalmological indications.

The first support for a functional role of the β_3 -adrenoceptor in the eye has been delivered by Geyer and colleagues, who suggested that this receptor subtype contributes to β -adrenergic relaxation of the bovine iris sphincter possibly via an increase in cellular cyclic adenosine monophosphate (cAMP) concentration (Geyer et al. 1998). The findings have been confirmed and extended by an independent study group reporting that β_2 - and β_3 -adrenoceptors mediate β -adrenergic relaxation responses in the bovine iris sphincter and ciliary muscle (Topalkara et al. 2006). Moreover, the latter study also demonstrated agonist-induced elevations of cAMP and cyclic guanosine monophosphate (cGMP) in these muscle preparations. However, both studies were largely based on the agonist BRL 37344, a compound which can activate not only β_3 - but also β_2 -

adrenoceptors (Mori et al. 2010), and indeed, part of the BRL 37344 effects were inhibited by a β_2 -adrenoceptor antagonist (Topalkara et al. 2006). Moreover, the cGMP effects apparently involved an intermediary stimulation of NO synthase.

In immunohistochemical studies that used antibodies directed against individual β -adrenoceptor subtypes, β_3 -adrenoceptor expression has been demonstrated in stratified squamous epithelial cells and goblet cells from human conjunctiva (Diebold et al. 2001; Enriquez de Salamanca et al. 2005). In contrast, no β_3 -adrenoceptor immunoreactivity was detected in mouse and rat conjunctiva (Diebold et al. 2001). These findings suggest that conjunctival β_3 -adrenoceptor expression is species dependent. However, the expression data obtained with “selective” antibodies have to be interpreted with caution, since a variety of antibodies raised against G protein-coupled membrane receptors, including β_3 -adrenoceptors, was shown to exhibit only low target selectivity (Pradidarcheep et al. 2009; Cernecka et al. 2012). Moreover, some of these antibodies exhibit specificity between rodent and human β_3 -adrenoceptors, which may lead to false-positive conclusions about species differences.

A recent study by Oikawa and colleagues reported that activation of β_3 -adrenoceptors by intravitreal injection of the β_3 -adrenoceptor agonist CL 316,243 protects against retinal damage induced by *N*-methyl-D-aspartate (NMDA) (Oikawa et al. 2012). Overactivation of NMDA receptors on retinal neurons mediates damage to these cells mainly through excessive calcium entry and subsequent activation of calcium-dependent intracellular signalling pathways eventually resulting in cell death. This mechanism is thought to be involved in various pathological conditions of the retina and optic nerve, such as retinal ischemia, diabetic retinopathy, and glaucoma (Shen et al. 2006). Thus, β_3 -adrenoceptor agonists might become an attractive pharmacological tool to treat these diseases. However, it is unknown at present how β_3 -adrenoceptors may influence

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NMDA-induced excitotoxicity. So far, no evidence for an expression of β_3 -adrenoceptors on retinal neurons has been provided. Studies in mice that used an antibody directed against the β_3 -adrenoceptor presented its specific localization on blood vessels (Ristori et al. 2011). It has been proposed that an increase in oxygen supply due to increased retinal perfusion induced by activation of β_3 -adrenoceptors may facilitate neuron survival in the retina after NMDA exposure (Oikawa et al. 2012), based on studies reporting that activation of β_3 -adrenoceptors evokes vasodilation in retinal arterioles (Mori et al. 2010). It remains, however, to be determined whether β_3 -adrenoceptor agonists exert protective effects on retinal neurons directly. Furthermore, it needs to be confirmed whether the findings of Oikawa et al. can be extrapolated to humans, because the β_3 -adrenoceptor agonist CL 316,243 used in this study was shown to be effective and selective in rodents (Arch et al. 1984; Bloom et al. 1992), but with less selectivity for human β -adrenoceptor subtypes (Baker 2005).

In vivo studies in rats, in which β_3 -adrenoceptor-selective agonists and antagonists had been administered intravenously, reported that activation of β_3 -adrenoceptors evokes dilation of retinal arterioles with only mild systemic cardiovascular effects and that β_3 -adrenoceptors are involved in retinal vasodilation responses to adrenaline (Mori et al. 2010, 2011). Of note, these are some of the few studies, in which based on the use of highly selective antagonists, such as L-748,337, the involvement of β_3 -adrenoceptors has been demonstrated with high reliability. Moreover, the findings of Mori et al. suggest that β_2 -adrenoceptor-mediated vasodilation undergoes desensitization in diabetic rats, whereas β_3 -adrenoceptor-mediated vasodilation in retinal vessels does not (Mori et al. 2010). Based on these findings, selective β_3 -adrenoceptor agonists may become useful to improve retinal perfusion in ischemic diseases with minor cardiovascular side effects. It remains, however, to be confirmed whether these findings can be extrapolated to humans.

In this issue, Dal Monte and colleagues report that β_3 -adrenoceptor expression is upregulated by hypoxia in isolated retinas of C57BL/6J mice, a finding which is in line with previous reports in mouse models of retinopathy of prematurity (Ristori et al. 2011; Chen et al. 2012; Dal Monte et al. 2012b). Moreover, by the use of different β_3 -adrenoceptor-selective agents, such as the agonist BRL 37344, and the antagonists SR59230A and L-748,337 as well as β_3 -adrenoceptor siRNA, the study demonstrates that activation of β_3 -adrenoceptors by hypoxia mediates VEGF release via NO synthase activation (Dal Monte et al. 2013). A previous study of the same group performed in a mouse model of oxygen-induced retinopathy of prematurity using C57BL/6 mice has reported that the β -adrenoceptor antagonist propranolol was effective in protecting against pathologic

retinal neovascularization and blood barrier breakdown, presumably via suppression of β -adrenoceptor-mediated VEGF overexpression (Ristori et al. 2011). It has, however, to be noted that another study group using the same disease model, but the 129S6/SvEvTac mouse strain and different methods of evaluating retinopathy, did not show any effect of propranolol on VEGF expression and on pathologic neovascularization (Chen et al. 2012). Moreover, propranolol has only low affinity for β_3 -adrenoceptors (Baker 2005), indicating that these data may be explained by β_1 - or β_2 -adrenoceptor engagement. Thus, a reevaluation of the findings in this ischemic model is needed.

Another open question is how β_3 -adrenoceptors may mediate VEGF release, because their expression has been demonstrated in retinal vessels and neovascular tufts, but not on retinal glial cells (Ristori et al. 2011), which are responsible for the major part of VEGF secretion (Pierce et al. 1995, 1996). The present study by Dal Monte et al. provides possible pathways by which β_3 -adrenoceptor agonists/antagonists may modulate VEGF release through NO in vascular endothelial cells and surrounding cells, including neuroglial cells (Dal Monte et al. 2013). This work expands on previous work of the same group on hypoxia in the retina (Dal Monte et al. 2012a) and other tissues (Dal Monte et al. 2011).

It remains to be established whether the findings of Dal Monte et al. can be extrapolated to the human retina. Interestingly, a role for β_3 -adrenoceptors in the control of cell proliferation and migration has been demonstrated in human retinal endothelial cells (Steinle et al. 2003), and in invasion, proliferation, and elongation in human choroidal endothelial cells (Steinle et al. 2005). Additional studies in human cell and tissue cultures are needed to extend these findings. The study of Dal Monte et al. suggests that blockade of β_3 -adrenoceptors may be beneficial in treating hypoxic/ischemic retinal diseases (Dal Monte et al. 2013). However, since other studies have shown that activation of β_3 -adrenoceptors mediates vasodilation in retinal arterioles and protects against NMDA-induced retinal damage, blockade of these receptors might even promote ischemia and neuronal cell death in vivo. On the other hand, β_3 -adrenoceptors can couple to multiple signalling pathways, and a given compound can be a strong agonist for one, but a much weaker agonist or even antagonist for another signalling response, a phenomenon called ligand-directed signalling or biased agonism (Evans et al. 2010). A β_3 -adrenoceptor ligand blocking direct adverse effects on the retina and simultaneously promoting vasodilatation of retinal blood vessels may prove interesting in this regard but has not yet been identified. If such a drug is not found, it remains to be determined whether the direct retinal or the indirect effects via the retinal blood vessels dominate in vivo.

In conclusion, there is evidence that β_3 -adrenoceptors play a functional role in regulating blood flow, responses

to hypoxia, and in protection from NMDA receptor-mediated excitotoxicity in the retina. Hence, from a clinical point of view, selective targeting of β_3 -adrenoceptors may become useful to treat retinal hypoxia/ischemia and to protect neurons from cell death. We propose that the role of β_3 -adrenoceptors in regulating the effects of retinal hypoxia/ischemia and in NMDA receptor-mediated cell death may need further validation *in vivo*. Moreover, findings in experimental animals need to be extended to humans.

Conflict of interest AG and TB do not report a conflict of interest. In the β_3 -adrenoceptor field, MCM has received research support and/or consultancy honoraria from AltheRX and Astellas in recent years; he, currently, is an employee of the Boehringer Ingelheim.

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