Cross-regulation between cardiac muscarinic acetylcholine receptors and β -adrenoceptors: lessons for use of knock-out mice

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The two arms of the autonomic nervous system, the sympathetic and the parasympathetic nervous system, typically elicit opposite reactions in the mammalian body. In the heart, this often occurs via β -adrenoceptors on the one and muscarinic acetylcholine receptors on the other hand. Thus, the balance between these two receptor systems can be important for the cell function and specifically contractile tone. However, these two receptor systems not only co-exist but also interact with each other acutely and chronically. Such acute interaction appears obvious when β -adrenoceptors and muscarinic receptors of the M_2 -like subfamily are considered, i.e., M_2 and M_4 receptors, as their prototypical signaling pathways include enhanced and reduced cyclic AMP formation, respectively. That such interaction also occurs between subtypes of β -adrenoceptors and those of M_3 -like muscarinic receptors, i.e., M_1 , M_3 , and M_5 receptors, is less obvious but nevertheless consistently found, e.g., in the urinary bladder (Andersson and Arner 2004).

Perhaps even more important for disease states or chronic treatment is the long-term interaction between these two systems, and a paper in the current issue of the journal (Benes et al. 2013) exemplifies this based on M_2 receptor knockout mice. In their study, Benes et al. used a general knockout for M_2 cholinergic receptors in mice and observed that

this was not accompanied by larger changes in hemodynamic parameters. Echocardiography revealed that left ventricular systolic and diastolic function was nearly unchanged, so that left ventricular end-systolic and end-diastolic diameters as well as fractional shortening, mitral flow characteristics, and maximal velocity in the left ventricular outflow tract were similar between wild-type and knockout mice. The explanation given by the results is that the knockout of the cardioinhibitory M_2 cholinergic receptors is counter-regulated by a decrease in β_1 or β_2 adrenoceptor density as assessed, e.g., by radioligand binding. Importantly, the catecholamine levels did not differ between the two groups of mice, indicating that the counter-regulation is not on the level of mediator release. However, it needs to be kept in mind that this is limited to the circulating catecholamines as the local release was not measured. Thus, the counter-regulation seems to be focussed on the receptor level. Whether additional changes on the G-protein level, small G-proteins or receptor-effector coupling may also contribute, remains to be established.

The basic phenomenon that regulation of either muscarinic receptors or β -adrenoceptors is accompanied by regulation of the other in the opposite direction has been known for more than two decades, specifically based on studies in the heart. This has been shown in a variety of experimental settings, e.g., with sympathetic denervation-sensitizing responses to catecholamines and concomitantly reducing muscarinic receptor expression in dog heart (Vatner et al. 1985), chronic β_1 antagonist treatment desensitizing M_2 muscarinic receptor function in human (Motomura et al. 1990) and rat heart (Borst et al. 1997; Marquetant et al. 1992), chronic isoprenaline treatment enhancing negative inotropic effects of carbachol in rat heart (Mende et al. 1992) or chicken cardiomyocytes (Reithmann et al. 1992), or with chronic muscarinic agonist treatment enhancing β -adrenergic function in rat heart (Eschenhagen et al. 1996; Reithmann and Werdan 1995). Such opposite regulation

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may differentially affect subtypes within a receptor subfamily (Aprigliano et al. 1997). Moreover, functional opposite regulation may not only occur at the level of receptor expression but may also involve various more distal levels including expression of G-protein α -subunits (Borst et al. 1997; Eschenhagen et al. 1996; Mende et al. 1992; Reithmann et al. 1992; Reithmann and Werdan 1995) or even more distal components of their signaling cascade such as NO formation (Balligand et al. 1993). Why such opposite regulation differentially affects receptor subtypes and why it may involve different levels important for receptor function in different setting largely remains to be explored.

However, it needs to be noted that such opposite regulation of muscarinic receptors and β -adrenoceptors has not been observed under all experimental conditions, as both a lack of opposite regulation (Matthews et al. 1996; Quist et al. 1994) and even a concomitant regulation of both systems in the same direction have been observed, e.g., after aortic banding in rat heart (Mondry et al. 1995) after 6-hydroxydopamine treatment in rat papillary muscle (Chen and Wince 1988) or after concanavalin A treatment in cultured rat cardiomyocytes (Rocha-Singh et al. 1991). Why opposite regulation of muscarinic receptors and β -adrenoceptors is present under some but not other conditions, remains largely unclear.

Against this background, it is neither obvious nor surprising that Benes et al. (2013) now report that in mice with systemic knockout of M_2 receptors considerable cross-regulation of M_3 muscarinic but also of β_1 and β_2 adrenoceptors occurs, which has implications for heart rate regulation by the autonomic nervous system. However, this study reinforces the established but often neglected phenomenon that life-long systemic knockout of one receptor may lead to changes in receptor systems functionally opposing the lacking receptor; such cross-regulation may complicate the interpretation of findings from knockout animals as it may at least partly conceal the expected physiological consequences of receptor loss.

Interestingly, in older pharmacological experiments such a reduction of M-cholinoceptors was induced by cholinesterase-inhibition using diisopropylfluorophosphate, which resulted in an early (24–48 h) increase in both muscarinic cholinceptors and β -adrenoceptors followed by a decrease in the density of both receptor types to about half of the control value on day 5 (Myslavecsek et al. 1996). Similar to the observations presented actually by Benes et al. (2013), these authors also found only very small alterations of cardiac hemodynamic parameters which was exalined by the parallel regulation of cardio-inhibitory and cardiostimulatory receptors. In contrast, in patients with combined sympathetic and parasympathetic lesions, it was shown that these patients were irresponsive to atropine but exhibited supersensitivity to isoprenaline (Man in't Veld and Schalekamp 1982). However, the situation might be

even more complex since, e.g., the vasoactive intestinal polypeptide (VIP) also is co-regulated. Thus, chronic antimuscarinic treatment with atropine affects VIP synthesis in rat heart atria and modifies heart rate responses (Kuncová et al. 2008).

In conclusion, cross-regulation between adrenoceptors and cholinceptors has been demonstrated in many studies. However, it remains to be studied why it differentially involves receptor subtypes, why it occurs at different levels of the receptor signaling cascade, and why it does not occur under all experimental conditions. In a broader sense, such studies are a reminder that life-long knockout of one gene can lack expected or have unexpected consequences due to cross-regulation. On the other hand, studies based on systemic knockout mice, e.g., for the M_2 receptor (Ehlert et al. 2007) and studies based on highly M_2 selective antagonists (Witte et al. 2011) have yielded consistent findings on the interaction between the muscarinic and the β -adrenoceptor system, e.g., in the urinary bladder.

Conflict of interest MCM is an employee of Boehringer Ingelheim Pharma GmbH & Co. KG. DS and SvS have conflict of interest to declare.

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