

Regulation of Adrenoceptors and Muscarinic Receptors in the Heart

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Abstract. Receptor activation results in homologous regulation and can also affect other types of receptors (a process that has been reported to heterologous regulation). Heart cells express subtypes of muscarinic receptors and adrenoceptors, almost antagonistic in their action (M_2 muscarinic receptors and β_1 -adrenoceptors). Therefore, they provide an excellent model of heterologous regulation. Moreover, the minor subtypes of adrenoceptors and muscarinic receptors have been identified in the heart cells. The physiological significance of the minor subtypes is now under keen investigation and their function can be considered as complementary to the major subtypes. Taken together, it seems that the minor subtypes may play an important role in the receptor-heart function homeostasis and that heterologous regulation seems to exist in many heart receptor types and in the above mentioned pair of receptors.

Key words: Adrenoceptors — Muscarinic receptors — Homologous regulation — Heterologous regulation — Heart

Introduction

Neurotransmitter receptors are highly dynamic protein structures, which are able to change the level of a transmitted signal in a few fractions of a second. The sensitivity to extracellular messengers can be modified not only simply as a consequence of massive activation of an appropriate receptor type (homologous regulation, see Morris and Malbon 1999), but also by the activation or inhibition of other receptors present in the cell (heterologous regulation, see Selbie and Hill 1998; Bünemann et al. 1999; Cordeaux and Hill 2002). Indirect effects of receptor activation can be comprehended as “fine-tuning” of the receptor-mediated signal (Selbie and Hill 1998; Cordeaux and Hill 2002). When we speculate about receptor systems as mutually interconnected regulatory systems, we have to stress that one of the most

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impressive examples of receptor cross-regulation is the heart tissue. Myocardial cells are exceptional in that they are all equipped with a pair of neurotransmitter receptors which have clearly defined antagonistic effects on their basic physiological functions, i.e. M_2 muscarinic receptors (M_2MR) and β_1 -adrenoceptors (β_1 -AR). Moreover, cardiomyocytes express other subtypes of adrenoceptors (AR) and muscarinic receptors (MR) in addition to the above mentioned pair of receptors of the autonomic nervous system, which are of minor significance or of uncertain physiological relevance. Despite the fact that some functions are not still fully understood, we would like to illustrate intriguing physiological dynamics of these G protein-coupled receptors (GPCRs) changing the level of the transmitted signal not only under conditions of classical “heterologous regulation” but also when changes of ionic flow through the membrane occur.

Muscarinic receptors and adrenoceptors expressed in heart cells

Myocardial cells express a spectrum of MR and AR, which belong to a large family of seven transmembrane-spanning proteins, namely G-protein coupled receptors (GPCRs). The above mentioned subtypes (M_2MR and β_1 -AR) are not the sole subtypes expressed in cardiomyocytes, although they are more relevant in their physiological function than the others. In the following paragraphs, the subtypes of AR and MR expressed in the heart will be shortly mentioned.

Adrenoceptors

Heart cells express the β_1 and β_2 subtype of AR, but the relative density of β_1 -AR is much higher than that of the β_2 -AR subtype. Both subtypes stimulate adenylyl-cyclase (AC). The principal function of β -AR is to mediate positive inotropy and chronotropy (Brodde and Michel 1999; Kaumann and Molenaar 1997). The other important function of β -AR (especially of β_1 -AR) concerns stimulation of Ca^{2+} channels (*via* proteinkinase A (PKA) or directly by G protein ($G_s\alpha$) binding).

The occurrence of other β -AR subtype, β_3 -AR and its mRNA has previously been described in heart ventricles and atria (for review see Gauthier et al. 2000), with cardioinhibitive function mediated *via* G_i proteins, although their existence has been challenged by other researchers (Kaumann and Molenaar 1997). Moreover, β_3 -AR have shown substantial variable expression across species.

The last subtype of β -AR, which has been discovered in the heart, is the fourth subtype, also called atypical cardiostimulatory β -AR, till the year before last assumed as putative β_4 -AR (Kaumann and Molenaar 1997). These binding sites are unique in their behavior and are now considered as an atypical state of the β_1 -AR (Granneman 2001). Changes in the “propranolol/bupranolol-insensitive form of β_1 -AR” (Brodde and Michel 1999) followed a very similar pattern as those observed for β_1 -AR. But, under certain conditions, it can be questioned since we have shown recently (Mysliviček and Tuček 2002) that putative β_4 -AR have been changed reciprocally to β_1 -AR by hydrocortisone treatment.

Heart cells also express α_1 -AR (Brodde and Michel 1999). To date, the expression of subtypes (α_{1A} and α_{1B}) and their functional consequences are not fully understood (for review see Table 1) and species differences may exist. Functionally, α_1 -AR are able to increase cardiac contractility and excitability, and they also alter heart metabolism and they can (similarly to β -AR) induce cell growth (Zimmer 1997).

Muscarinic receptors

The M_2 subtype mostly represents MR in the mammalian myocardium. The function of MR is opposite to those of β -AR (i.e. negative inotropy and chronotropy). M_2 receptors are coupled to the G_i protein (they inhibit AC), but a potent activation of the receptor permits interaction of the receptor with G_q and activation of phospholipase C (PLC) (for review see Dhein et al. 2001). A further consequence of MR activation is its influence on ionic flow through the membrane *via* activation or inhibition of appropriate ion channels. M_2 MR inhibit Ca^{2+} channels indirectly *via* decreased production of cAMP and lower PKA activity; the main result of MR activation mediated *via* ion channels is the activation of inwardly rectifying acetylcholine sensitive potassium channels (K_{ACH}). Activation of K_{ACH} leads to hyperpolarization and negative inotropy in the atria. In addition, these channels also participate in negative chronotropy. Another situation occurs in a pacemaker (sinatrial node), where the negative chronotropy is considerably affected by muscarinic inhibition of adenylylase.

The evidence that M_2 receptors are not the only subtype of MR in the heart is gaining support, but the problem of identification and quantification has not been satisfactorily solved. Among all non- M_2 receptors identified pharmacologically and/or electrophysiologically in the heart up to now (M_1 , M_3 , M_4 , see Brodde and Michel 1999), the M_1 subtype (cardiostimulating, coupled to the G_q /PLC, see Table 1) is the best documented (M_1 mRNA detection by single-cell reverse transcriptase-polymerase chain reaction (rT-PCR), Colecraft et al. 1998), although recent data challenged this hypothesis (Hamilton et al. 2001).

A simplified view on MR and AR in heart function is presented in Table 1.

Further consequences of opposite roles of the above mentioned receptors (muscarinic *vs.* adrenergic) lie in their effects on important factor of excitation-contraction coupling – ryanodine receptors (RYR). These “receptors” – better Ca^{2+} sensitive channels – are the subjects of phosphorylation by PKA as a result of β -AR stimulation. Moreover, they can also be activated *via* enhanced level of intracellular Ca^{2+} . Therefore they can be considered as one of the “targets of opposite action” of adrenoceptors *vs.* muscarinic receptors. Explanation of cardiostimulative effect of α_1 -AR and M_1 MR thus gains new importance.

Homologous regulation

Massive agonist activation of GPCRs leads rapidly to attenuation of the GPCR-mediated response as a consequence of receptor phosphorylation by G protein-

Table 1. Receptors, G proteins, effectors and their effects in the heart

receptor	β_1 -adrenoceptors	β_2 -adrenoceptors	β_3 -adrenoceptors	β_4 -adrenoceptors (atypical state of β_1 -adrenoceptor)	α_1 -adrenoceptors	M ₂ muscarinic receptors	minor muscarinic subpopulation ? M ₁ ? muscarinic receptors
occurrence	atria ventricles	atria ventricles	atria ? ventricles	atria ventricles	atria ventricles	atria ventricles	ventricles
G protein	G _s	G _s , G _i	G _i	G _s	G _q	G _i	G _q
effectors	– adenylylcyclase stimulation – increase in Ca ²⁺ intra- cellular level (L-type Ca ²⁺ channel phos- phorylation, activation of ryanodine receptor)	– adenylylcyclase stimulation	– adenylylcyclase inhibition – decreased Ca ²⁺ channel phosphoryla- tion ? – nitric oxide synthesis	adenylylcyclase ?	– phospholipase C β activation – phospholipase D activation – K _t channel inhibition – K _{ir} channel inhibition – K _{ACh} channel inhibition – Ca ²⁺ channel activation ?	– adenylylcyclase inhibition (SA node) – K _{ACh} channel activation	– phospholipase C β activation – inhibition of K ⁺ channels ?
effect	cardiostimulation	cardiostimulation	cardioinhibition	cardiostimulation	↑frequency ↑contactility ↑excitability cell growth	cardioinhibition	↑frequency

K_t, potassium transient outward; K_{ir}, potassium inward rectifier; K_{ACh}, acetylcholine sensitive potassium channel.

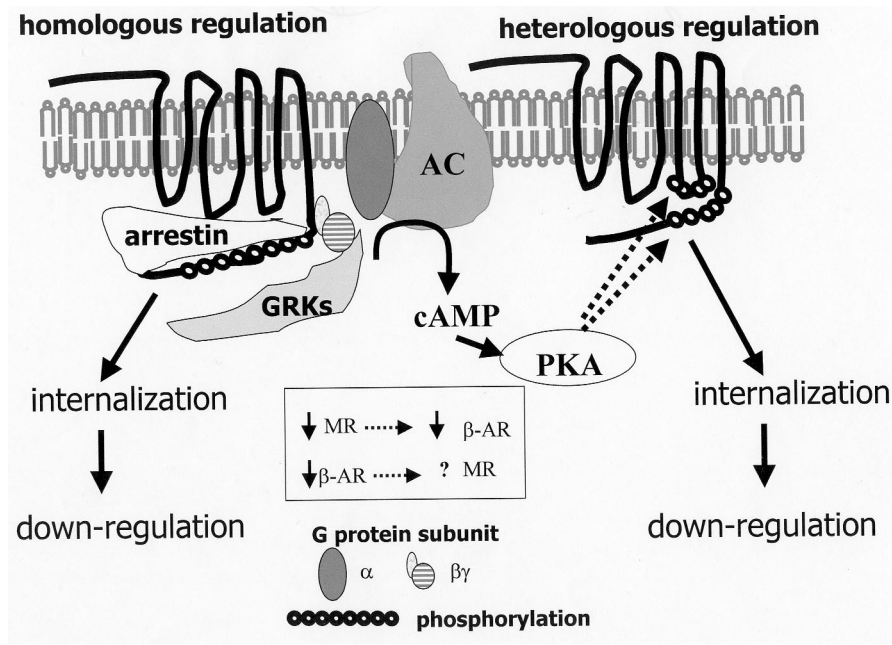


Figure 1. Schematic diagram of homologous and heterologous regulation. Homologous regulation is caused by phosphorylation *via* GRKs, heterologous regulation is caused *via* second messenger-dependent protein kinases (PKA, PKC). In case of homologous regulation the phosphorylated form of receptor is stabilized by arrestin. Insert: Effects of β -adrenoceptor (β -AR) activation on muscarinic receptors (MR) or vice versa.

coupled receptor kinases (GRKs), six members cloned so far – see Morris and Malbon 1999. Receptors phosphorylated by GRKs (see Figure 1), but not by second messenger-dependent protein kinases (i.e. PKA and PKC), can bind arrestins (four members – see Bünemann et al. 1999), which further prevent receptor-G protein interactions (Ferguson et al. 1997). Heart GPCRs may undergo phosphorylation on their C terminus (e.g. β_2 -AR) or on their third cytoplasmic loop (e.g. α_2 -AR, M_2 MR) – see Bünemann et al. 1999. The next event in the fate of phosphorylated receptors is targeting the receptors to vesicles (Tsao et al. 2001), a process that has been reported to internalization (see Figure 1). In spite of this, phosphorylation-independent receptor internalization may also occur. This process can proceed in receptor degradation that is called down-regulation of total receptor number. Furthermore, reversion of internalized receptors back to the cytoplasmic membrane is possible (i.e. resensitization).

Three types of vesicles are involved in the internalization process (for review see Hunyady et al. 2000):

- a) clathrin-coated vesicles (involved in internalization of most GPCRs; clathrin is a protein and forms triskelion with three heavy and three light chains),

- b) caveolae (involved in internalization of several GPCRs; caveolar structure is a lipid-based microdomain made up of caveolin protein and specific lipids), and
- c) non-coated vesicles (involved in internalization of some MR and β_2 -AR, the nature of which has not been elucidated yet).

The exact nature of the internalization process is still uncertain and molecules essentially required for association of receptors with vesicles (arrestins, dynamin, i.e. self-associating GTPase, and other adaptor proteins) are dubious. To date, it seems that there are at least three possible pathways of internalization:

- a) arrestin-, dynamin- and clathrin-dependent (example: β_2 -AR),
- b) arrestin-independent, dynamin-dependent (e.g. odd-numbered subtypes of MR and M_4 MR), and
- c) arrestin-independent, dynamin-independent (e.g. M_2 MR).

Alternatively (Morris and Malbon 1999), it is possible to view these processes as posttranslational regulation (protein phosphorylation, i.e. desensitization) and posttranscriptional regulation (destabilization of mRNA, i.e. down-regulation).

Heterologous regulation

Heterologous regulation among GPCRs is a process, whereby protein kinases (PKA, PKC) target all receptors despite whether they are occupied or vacant (see Figure 1). This is an important disparity between heterologous and homologous regulation (which is mediated *via* GRKs). Heterologous regulation can act either as an amplifier of the targeted receptor response or as its quencher. Cross-talk is well documented between G_i - and G_q -coupled receptors, G_s - and G_q -coupled receptors (for review see Cordeaux and Hill 2002; Hur and Kim 2002).

Moreover, not only receptor phosphorylation but also phosphorylation of $G\alpha_i$ protein has been suggested to play a role in regulating the inhibitory action of G_i protein. Phosphorylation of GPCRs *via* receptors with intrinsic tyrosine kinase activity is another way of “fine-tuning” of the GPCRs-mediated signal (Selbie and Hill 1998; Cordeaux and Hill 2002; Hur and Kim 2002).

Alternatively (Morris and Malbon 1999), cross-regulation can be comprehended both as posttranslational regulation (protein phosphorylation, i.e. desensitization), posttranscriptional regulation (destabilization of mRNA, i.e. down-regulation) and also as transcriptional regulation (when the regulation of targeted genes is involved).

The mechanisms of heterologous regulation are not fully understood yet. In many cases, heterologous regulation of receptors expressed in the cardiovascular system has been studied on cells with stable expression of receptors to autonomic system neurotransmitters (for review see Bünemann et al. 1999). This approach implies a question to what extent the results from these tissues can be extrapolated to the physiological conditions. Therefore, cross-regulation in cardiomyocytes will only be discussed further.

Heterologous regulation of MR and AR in the heart can be considered at two levels: a) as cross-regulation between this pair of receptors, and b) as regulation

of AR and/or MR by another physiological agent. We will pay attention to both above-mentioned levels.

Effect of muscarinic receptor activation on adrenoceptors

The pilot studies concerning this type of regulation appeared about twenty years ago (Watanabe et al. 1978; Limas and Limas 1985). Watanabe and colleagues employed the canine heart homogenates incubated with methacholine and found the changes in AR affinities to isoprenaline in the presence of GTP. Limas and Limas incubated enzymatically dissociated rat cardiomyocytes with carbachol and found time and concentration dependent decrease in β -adrenoceptors, which brought about redistribution of receptors from the membrane to cytosol. Similarly, a decrease of β -AR has been described in the long-term presence of muscarinic agonists in the incubation media in cell cardiomyocyte culture (Paraschos and Karliner 1994). Despite that, β -AR-mediated adenylyl cyclase activity was preserved (postreceptor-mediated adenylyl cyclase activity was augmented). Moreover, biphasic changes (initial increase followed by a decrease) in the β -AR (without changes in the heart rate) can be observed in the rat heart atria after cholinesterase inhibition (Myslivičėk et al. 1996), or after incubation of cardiomyocytes in culture with carbachol (Myslivičėk et al. 1998). On the other hand, Reithmann and Werdan (1995) noted no change in the number of β -AR on the surface of rat cardiomyocytes after 3 days of carbachol exposure.

It can be therefore summarized that MR stimulation in most cases decreases the number of β -AR. We can consider this regulation as the result of a mechanism by which a stable level of the signal into the cell can be maintained, i.e. this is a good example of “fine-tuning” in signal transduction. In other words: in spite of changes in the number of receptors, the pathways activated by them do not change significantly.

Effect of adrenoceptor activation on muscarinic receptors

The results concerning the *vice versa* regulation in the heart are not such explicit as the above mentioned. In pioneering work, Rosenberger et al. (1980) have found that isoprenaline decreased the affinity of MR. The activation of AR can lead to a decrease of MR both in adult animals and in cell cultures (Nomura et al. 1982; Myslivečėk et al. 1998; Garofolo et al. 2002). A decrease in the MR in culture cannot be a simple consequence of an activation of adenylylcyclase and protein kinase A, as it can be deduced from experiments with highly specific PKA inhibitors, adenylyl cyclase activators or cAMP nonhydrolyzable analogues (Myslivičėk et al. 1998). Matthews et al. (1996) have reported no change in MR protein but down regulation of M_2 mRNA level in different heart regions. On the other hand, Reithmann et al. (1992), Jackson and Nathanson (1995) and Myslivečėk et al. (1998) have found the increase in MR number after prolonged β -AR agonist exposure in cardiomyocytes.

Once again, the results are not explicit, although in other systems (cell lines transfected with M_2 and β_2 -AR (Lee and Fraser 1993) and human embryonal pneumocytes (Roussel et al. 1996) the same direction of receptor changes as in regulation of adrenoceptors by MR can be observed.

Taken together, the activation of both receptor types produces heterologous regulation, but the nature of these regulation have to be clarified yet. A simplified schema of homologous and heterologous regulation between these receptors is shown in the insert on Figure 1.

Regulation of muscarinic receptors and β -adrenoceptors by “permissive” hormones (i.e. *via* cytoplasmic receptors)

The main effect of cytoplasmic receptor activation is the influence on the genetic information *via* hormone response elements (HREs). When these sequences are present in GPCR genes (in the promoter region) it is possible to expect some effects on the expression of GPCRs (transcriptional activation or repression). For example, such elements have been demonstrated in β_2 -AR (Collins et al. 1991) and very recently (Tseng et al. 2001) in β_1 -AR, although regulatory units on the β_1 -AR gene differ from those on β_2 -AR.

β -AR can be influenced by steroid hormones (especially glucocorticoids) or by thyroid hormones. It has been reported (see Morris and Malbon 1999) that the decrease in β_1 -AR and the increase in β_2 -AR after dexamethasone treatment occurs in C6 glioma cells with spontaneous expression of these receptors, primarily due to a reduction of β_1 -AR transcription speed, as the total number of β -AR was not changed. The similar pattern of changes in the receptor number can be achieved in rat heart ventricles (increase in β_2 -AR, no change in β_1 -AR) treated by hydrocortisone (Mysliviček and Tuček 2002). On the contrary, this pattern of changes did not arise in rat heart atria. In this case both β_1 -AR and β_2 -AR increased. The total number of β -AR was augmented both in the atria and ventricles. Moreover, the α_1 -AR underwent a decrease followed by a transient increase in the atria but no change in the ventricles.

It is commonly conceded that thyroid hormones increase the number of β -AR (for review see Morris and Malbon 1999) and change the amount of α - and β -myosin heavy chain (β -MHC) in the heart.

On the contrary, changes in MR have as yet to be studied more deeply; the findings are different in various tissues (increase in the lung and thymocytes, decrease in bronchial smooth muscles). It seems that an increase in MR number can occur after glucocorticoid treatment in the heart atria and ventricles (Mysliviček and Tuček 2002).

Sex hormones enhance the density of autonomic nervous system receptors in the hearts of ovariectomized rats, but only if both progestins and estrogens were used. Hormones alone did not show these effects (Klangakalya and Chen 1988).

Activation of the thyroid receptor (*via* triiodothyronin) decreases MR density in intact myocardial cells and attenuates the effect of the muscarinic agonist (Waisberg and Shainberg 1992).

It can be summarized that the effects of permissive hormones on MR and AR are more complex than those produced by simple activation of AR or MR. On the other hand, glucocorticoid hormones produced more homogeneous responses (i.e. parallel changes) than thyroid hormones.

Changes in the number of G protein-coupled receptors (muscarinic receptors and β -adrenoceptors) following changes in ionic flow through the membrane

It has been described previously that GPCRs are able to change the conformation of ion channels and therefore activate or inhibit them. Moreover, enhanced GPCR activation and consequently ion channel phosphorylation can be the source of ion channel regulation. The possible mechanism can be similar to that described in GPCRs. An intriguing fact is that regulatory events can also be contrariwise: changes in ionic flow (or more accurately the effects of its changes – i.e. changes in intracellular ion concentrations, current flow) can change the receptor number. As it has been mentioned above, one from the important factors that can be targeted to both MR and AR is the intracellular calcium (Ca_i) level. Therefore, it is not surprising that changes in (Ca_i) level (caused for example by calcium channel blocker) can inhibit the spontaneous contraction of cultured cardiomyocytes and subsequently change the β -AR number (Disatnik and Shainberg 1992). Surprisingly, calcium channel blocker was able to affect the isoproterenol induced cAMP production. The changes in β -AR were the result of decreased receptor synthesis. Similarly, it is possible to find the changes in β -AR or MR after sodium channel activation/inhibition (see Table 2).

Conclusions

Receptor activation can also be a trigger of regulatory events. These events are not necessarily limited to the occupied receptor only, but may also affect other

Table 2. Impact of ionic flow changes on adrenoceptor and muscarinic receptor density

	impact of	effect	tissue	references
adrenoceptors	Ca ²⁺ channel blockers	β -AR decrease	cardiomyocytes in culture	Disatnik and Shainberg (1992)
	lidokain (Na ⁺ channel blocker)	up-regulation of β -AR	neonatal rat cardiomyocytes	Mizuki et al. (1994)
muscarinic receptors	batrachotoxin (Na ⁺ channel activator)	enhanced affinity to agonists	rat atria	Minton and Sokolovsky (1990)

receptor systems, which can be functionally synergistic or antagonistic to the first system. To date, it seems that such regulation can exist between all receptor types and the importance of this in the heart tissue is gradually being discovered. The mechanisms of receptor regulation are not clearly understood although the role of receptor phosphorylation in desensitization is evident and the changes in genetic information transmission processes are probably involved in receptor down- and up-regulation.

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References

- Brodde O. E., Michel M. C. (1999): Adrenergic and muscarinic receptors in the human heart. *Pharmacol. Rev.* **51**, 651—688
- Bünemann M., Lee K. B., Pals-Rylaarsdam R., Roseberry A. G., Hosey M. M. (1999): Desensitization of G-protein-coupled receptors in the cardiovascular system. *Annu. Rev. Physiol.* **61**, 169—192
- Colecraft H. M., Egamino J. P., Sharma V. K., Sheu S. S. (1998): Signaling mechanisms underlying muscarinic receptor-mediated increase in contraction rate in cultured heart cells. *J. Biol. Chem.* **273**, 32158—32166
- Collins S., Caron M. G., Lefkowitz R. J. (1991): Regulation of adrenergic receptor responsiveness through modulation of receptor gene expression. *Annu. Rev. Physiol.* **53**, 497—508
- Cordeaux Y., Hill S. J. (2002): Mechanisms of cross-talk between G-protein-coupled receptors. *Neurosignals* **11**, 45—57
- Dhein S., van Koppen C. J., Brodde O. E. (2001): Muscarinic receptors in the mammalian heart. *Pharmacol. Res.* **44**, 161—182
- Disatnik M. H., Shainberg A. (1992): Effects of calcium and calcium-channel blocker methoxyverapamil on the β -adrenoceptors in myocardial cells *in vitro*. *Biochem. Pharmacol.* **43**, 213—217
- Ferguson S. S., Zhang J., Barak J. S., Caron M. G. (1997): Pleiotropic role for GRKS and β -arrestins in receptor regulation. *News Physiol. Sci.* **12**, 145—151
- Garofolo M. C., Seidler J. F., Auman J. T., Slotkin T. A. (2002): β -adrenergic modulation of muscarinic cholinergic receptor expression and function in developing heart. *Am. J. Physiol. – Regul. Integr. C.* **282**, R1356—1363
- Gauthier C., Langin D., Balligand J. L. (2000): β_3 -adrenoceptors in the cardiovascular system. *Trends Pharmacol. Sci.* **21**, 426—431
- Granneman J. G. (2001): The putative β_4 -adrenergic receptor is a novel state of the β_1 -adrenergic receptor. *Am. J. Physiol. – Endocrinol. Met.* **280**, E199—202
- Hamilton S. E., Hardouin S. N., Anagnostaras S. G., Murphy V. C., Richmond K. N., Silva A. J., Feigl E. O., Nathanson N. M. (2001): Alteration of cardiovascular and neuronal function in M1 knockout mice. *Life Sci.* **68**, 2489—2493
- Hunyady L., Catt K. J., Clark A. J., Gáborik Z. (2000): Mechanisms and functions of AT₁ angiotensin receptor internalization. *Regul. Pept.* **91**, 29—44
- Hur E. M., Kim K. T. (2002): G protein-coupled receptor signalling and cross-talk. Achieving rapidity and specificity. *Cell. Signal.* **14**, 397—405

- Jackson D. A., Nathanson N. M. (1995): Subtype-specific regulation of muscarinic receptor expression and function by heterologous receptor activation. *J. Biol. Chem.* **270**, 22374—22377
- Kaumann A. J., Molenaar P. (1997): Modulation of human cardiac function through 4 β -adrenoceptor populations. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **355**, 667—681
- Klangakalya B., Chan A. (1988): Structure-activity relationships of steroid hormones on muscarinic receptor binding. *J. Steroid Biochem.* **29**, 111—118
- Lee N. H., Fraser C. M. (1993): Cross-talk between m1 muscarinic acetylcholine and β_2 -adrenergic receptors. cAMP and the third intracellular loop of m1 muscarinic receptors confer heterologous regulation. *J. Biol. Chem.* **268**, 7949—7957
- Limas C. J., Limas C. (1985): Carbachol induces desensitization of cardiac β -adrenergic receptors through muscarinic M₁ receptors. *Biochem. Biophys. Res. Commun.* **129**, 699—704
- Matthews J. M., Falck H. J., Molenaar P., Summers R. J. (1996): Chronic (-)isoprenaline infusion down-regulates β_1 - and β_2 -adrenoceptors but does not transregulate muscarinic cholinergic receptors in rat heart. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **353**, 213—225
- Minton A. P., Sokolovsky M. (1990): A model for the interaction of muscarinic receptors, agonists, and two distinct effector substances. *Biochemistry* **29**, 1586—1593.
- Mizuki T., Kobayashi H., Nakashima Y., Kuroiwa A., Izumi F. (1994): Lidocaine increases the number of β -adrenoreceptors in neonatal rat cardiocytes in culture. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **349**, 170—174
- Morris A. J., Malbon C. C. (1999): Physiological regulation of G protein-linked signaling. *Physiol. Rev.* **79**, 1373—1430
- Myslivičėk J., Tuček S. (2002): Plasticity of cardiac adrenergic and muscarinic receptors under hydrocortisone. *J. Physiol. (Paris)* **96**, 152—153
- Myslivičėk J., Trojan S., Tuček S. (1996): Biphasic changes of muscarinic and β -adrenergic receptors in rat heart atria during DFP treatment. *Life Sci.* **58**, 2423—2430
- Myslivičėk J., Lisá V., Trojan S., Tuček S. (1998): Heterologous regulation of muscarinic and beta-adrenergic receptors in rat cardiomyocytes in culture. *Life Sci.* **63**, 1169—1182
- Nomura Y., Kajiyama H., Segawa T. (1982): Alteration in sensitivity to isoproterenol and acetylcholine in the rat heart after repeated administration of isoproterenol. *J. Pharmacol. Exp. Ther.* **220**, 411—416
- Paraschos A., Karliner J. S. (1994): Receptor crosstalk: effects of prolonged carbachol exposure on beta 1-adrenoceptors and adenylyl cyclase activity in neonatal rat ventricular myocytes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **350**, 267—276
- Reithmann C., Werdan K. (1995): Chronic muscarinic cholinergic stimulation increases adenylyl cyclase responsiveness in rat cardiomyocytes by a decrease in the level of inhibitory G-protein alpha-subunits. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **351**, 27—34
- Reithmann C., Panzner B., Werdan K. (1992): Distinct pathways for beta-adrenoceptor-induced up-regulation of muscarinic acetylcholine receptors and inhibitory G-protein alpha-subunits in chicken cardiomyocytes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **345**, 530—540
- Rosenberger L. B., Yamamura H. I., Roeske W. R. (1980): The regulation of cardiac muscarinic cholinergic receptors by isoproterenol. *Eur. J. Pharmacol.* **65**, 129—130
- Rousell J., Haddad E. B., Mak J. C., Webb B. L., Giembycz M. A., Barnes P. J. (1996): β -adrenoceptor-mediated down-regulation of M2 muscarinic receptors: role of cyclic

- adenosine 5'-monophosphate-dependent protein kinase and protein kinase C. *Mol. Pharmacol.* **49**, 629—635
- Selbie L. A., Hill S. J. (1998): G protein-coupled-receptor cross-talk: the fine-tuning of multiple receptor-signaling pathways. *Trends Pharmacol. Sci.* **19**, 87—93
- Tsao P., Cao T., von Zastrow M. (2001): Role of endocytosis in mediating downregulation of G-protein-coupled receptors. *Trends Pharmacol. Sci.* **22**, 91—96
- Tseng Y. T., Stabila J. P., Nguyen T. T., McGonnigal B. G., Waschek J. A., Padbury J. F. (2001): A novel glucocorticoid regulatory unit mediates the hormone responsiveness of the beta1-adrenergic receptor gene. *Mol. Cell. Endocrinol.* **181**, 165—178
- Waisberg M., Shainberg A. (1992): Characterization of muscarinic cholinergic receptors in intact myocardial cells *in vitro*. *Biochem. Pharmacol.* **43**, 2327—2334
- Watanabe A. M., McConnaughey M. M., Strawbridge R. A., Fleming J. W., Jones L. R., Besch H. R. (1978): Muscarinic cholinergic receptor modulation of β -adrenergic receptor affinity for catecholamines. *J. Biol. Chem.* **253**, 4833—4836
- Zimmer H. G. (1997): Catecholamine-induced cardiac hypertrophy: significance of proto-oncogene expression. *J. Mol. Med.* **75**, 849—859

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