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REVIEW

Are the pharmacology and physiology of α_2-adrenoceptors determined by α_2-heteroreceptors and autoreceptors respectively?

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α_2-Adrenoceptors are important mediators of physiological responses to the endogenous catecholamines noradrenaline and adrenaline. In addition, α_2-adrenoceptors are pharmacological targets for the treatment of hypertension, sympathetic overactivity and glaucoma. α_2-Adrenoceptors are also targeted to induce sedation and analgesia in anaesthesia and intensive care. α_2-Adrenoceptors were first described as presynaptic receptors inhibiting the release of various transmitters from neurons in the central and peripheral nervous systems. In addition to these presynaptic neuronal receptors, α_2-adrenoceptors were also identified in many non-neuronal cell types of the body. Gene-targeting in mice provided a comprehensive assignment of the physiological and pharmacological functions of these receptors to specific α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor subtypes. However, the specific cell types and signalling pathways involved in these subtype-specific α_2-adrenoceptor functions were largely unexplored until recently. This review summarizes recent findings from transgenic mouse models, which were generated to define the role of α_2-adrenoceptors in adrenergic neurons, that is, α_2-autoreceptors, versus α_2-adrenoceptors in non-adrenergic neurons, termed α_2-heteroreceptors. α_2-Autoreceptors are primarily required to limit release of noradrenaline from sympathetic nerves and adrenaline from adrenal chromaffin cells at rest. These receptors are desensitized upon chronic activation as it may for instance occur due to enhanced sympathetic activity during chronic heart failure. In contrast, pharmacological effects of acutely administered α_2-adrenoceptor agonist drugs essentially require α_2-heteroreceptors in non-adrenergic neurons, including analgesia, sedation, hypothermia and anaesthetic-sparing as well as bradycardia and hypotension. Thus a clear picture has emerged of the significance of auto- versus heteroreceptors in mediating the physiological functions of α_2-adrenoceptors and the pharmacological functions of α_2-adrenoceptor agonist drugs respectively.

Abbreviations
Dbh, dopamine-β hydroxylase; GRK2, G-protein receptor kinase 2

Introduction

The α_2-adrenoceptor family comprising α_{2A}-, α_{2B}- and α_{2C}-subtypes was identified by pharmacological (Cheung *et al.*, 1982; Latifpour *et al.*, 1982; Summers *et al.*, 1983; Feller and Bylund, 1984; Neylon and Summers, 1985) and molecular cloning techniques (Kobilka *et al.*, 1987; Regan *et al.*, 1988; Lomasney *et al.*, 1990). All three α_2-adrenoceptor subtypes are coupled to G-proteins of the $G\alpha_{i/o}$ family and therefore share general signalling properties (Limbird, 1988). α_2-Adrenoceptors were first discovered as presynaptic receptors in neurons inhibiting the exocytosis of several neurotransmitters (for review, see Starke, 2001). Depending on the location of the α_2-adrenoceptor and the neurotransmitter that is modulated by these receptors, the terms 'autoreceptors' and 'heteroreceptors' were introduced. 'Autoreceptors' refers to α_2-adrenoceptors, which are located in the presynaptic membrane of adrenergic neurons, thus inhibiting the exocytosis of their own neurotransmitters, noradrenaline or adrenaline, as part of a negative feedback loop (Starke, 2001).

Since noradrenaline-containing vesicles of sympathetic nerves also store several co-transmitters, including neuropeptide Y and ATP, the release of these neurotransmitters is also suppressed (Burnstock, 2006). In contrast to autoreceptors, 'heteroreceptors' are presynaptic release-modulating receptors, which are not activated by the neurotransmitter, which is synthesized by the neuron on which they are located (Starke, 2001).

In vitro studies confirmed that all three α_2-adrenoceptor subtypes are able to inhibit endogenous transmitter release from postganglionic sympathetic neurons and brain noradrenergic neurons (Trendelenburg *et al.*, 2003). α_2-Adrenoceptors are currently among the best characterized inhibitory autoreceptors (Starke *et al.*, 1975; Langer, 1980; Boehm and Huck, 1997; Hein *et al.*, 1999; Starke, 2001; Gilsbach and Hein, 2008) and are predominantly recognized for this function. They are however also expressed as 'heteroreceptors' on non-adrenergic neurons in the peripheral and central nervous system (Starke, 1977) and they can inhibit the release of many neurotransmitters including serotonin (Mongeau *et al.*, 1998; Jackisch *et al.*, 1999), GABA (Forray *et al.*, 1999; Zha *et al.*, 2007; Philbin *et al.*, 2010) and dopamine (Millan *et al.*, 2000).

α_2-Adrenoceptor subtypes

Apart from the endogenous agonists adrenaline and noradrenaline several synthetic ligands are available, including the clinically used α_2-adrenoceptor agonists brimonidine, clonidine, dexmedetomidine, moxonidine, medetomidine und rilmenidine (Cambridge, 1981; Kallio *et al.*, 1989; Bylund *et al.*, 1994; Fairbanks *et al.*, 2009). *In vivo* these α_2-adrenoceptor agonists elicit a wide range of effects including hypotension and bradycardia, analgesia, hypothermia, sedation, hypnosis and anaesthetic-sparing (Hoefke and Kobinger, 1966; MacMillan *et al.*, 1996; Lakhlani *et al.*, 1997; Kamibayashi and Maze, 2000; Scholz and Tonner, 2000; Maze *et al.*, 2001; Lahdesmaki *et al.*, 2003; Knaus *et al.*, 2007b; Sanders and Maze, 2007a). However, some of these effects limit the clinical applicability of current non-subtype-selective α_2-adrenoceptor agonists. Especially the sedative and cardiovascular effects are often not tolerable and prevent the application of α_2-adrenoceptor agonist for other therapeutic indications.

It was the hope that the spectrum of clinical effects of α_2-adrenoceptor agonists could be separated by developing subtype-selective ligands. While such substances have recently become available (Crassous *et al.*, 2007a; Gentili *et al.*, 2007; Sallinen *et al.*, 2007) studies in mice with ablation of the individual α_2-subtypes clearly show that only a few functions rely on a single subtype. For example, α_{2B}-adrenoceptors play an important role in development of the placenta (Philipp *et al.*, 2002; Muthig *et al.*, 2007) and the lung (Haubold *et al.*, 2010) and they regulate vascular tone (Link *et al.*, 1996). α_{2C}-Adrenoceptors were identified as the major feedback receptors of adrenaline release from chromaffin cells in the adrenal medulla (Brede *et al.*, 2002; 2003; Gilsbach *et al.*, 2007). However, the majority of physiological and pharmacological functions of α_2-adrenoceptors were attributed to the α_{2A}-subtype. The inhibition of insulin release depends, for example, on α_{2A}-adrenoceptors in pancreatic islets (Gribble, 2010; Rosengren *et al.*, 2010). Recent reports show that the facilitation of working memory is linked to neuronal α_{2A}-adrenoceptors in the prefrontal cortex (Wang *et al.*, 2007). In addition, the α_{2A}-subtype mediates hypotension, bradycardia and modulation of the baroreflex sensitivity (MacMillan *et al.*, 1996; Lakhlani *et al.*, 1997; Niederhoffer *et al.*, 2004) and also sedation and hypnosis (MacMillan *et al.*, 1996; Wang *et al.*, 2007).

Despite the comprehensive knowledge about the functional role of individual α_2-adrenoceptor subtypes that was mostly derived from gene-targeted mouse models, information about the precise cellular localization and signalling pathways engaged by α_2-adrenoceptors has been lacking until recently. Thus the present review primarily focuses on new studies in transgenic mouse models determining the significance of α_2-auto- versus heteroreceptors in physiology and pharmacology. In addition to these neuronal auto- and heteroreceptors, α_2-adrenoceptors in non-neuronal cells have essential roles in the body. Comprehensive reviews about α_2-adrenoceptors in non-neuronal cells have been published recently (Kable *et al.*, 2000; Guimaraes and Moura, 2001; Hein, 2001; Philipp and Hein, 2004; Gilsbach and Hein, 2008; Gyires *et al.*, 2009).

Transgenic dissection of α_{2A}-autoreceptors versus α_{2A}-heteroreceptors

We have recently generated a transgenic model to dissect physiological and pharmacological functions of α_{2A}-autoreceptors versus α_{2A}-heteroreceptors (Gilsbach *et al.*, 2009). In this transgenic model the dopamine-β hydroxylase (Dbh) promoter was utilized to drive expression of α_{2A}-adrenoceptors in adrenergic neurons (Figure 1). Previous studies showed that genes under control of the Dbh promoter are selectively expressed in adrenergic neurons (Mercer *et al.*, 1991; Hoyle *et al.*, 1994). In order to obtain mice, which only express α_{2A}-adrenoceptors as autoreceptors in adrenergic neurons, these transgenic mice were crossed with $\alpha_{2A/C}$-knockout animals and are thus termed 'Dbh-α_{2A}' in this review (Gilsbach *et al.*, 2009; 2010). An additional deletion of the α_{2B}-adrenoceptor gene was not possible due to embryonic lethality of $\alpha_{2A/B/C}$-knockout mice (Philipp *et al.*, 2002; Knaus *et al.*, 2007a).

The Dbh-α_{2A} mice were extensively characterized to confirm the correct expression pattern and function of the transgenic α_{2A}-autoreceptor. In these mice α_{2A}-adrenoceptor mRNA was expressed adrenergic neurons in the central and peripheral nervous system, including *locus coeruleus* as well as stellate and superior cervical ganglia (Gilsbach *et al.*, 2009; 2010). Using receptor autoradiography and immunohistochemistry receptor protein was detected in brain regions with known adrenergic innervation like the *lacunosum moleculare layer* of the hippocampus (Gilsbach *et al.*, 2009). Immunoelectron microscopy indicated transgenic α_{2A}-autoreceptors in plasma membranes of vesicle-

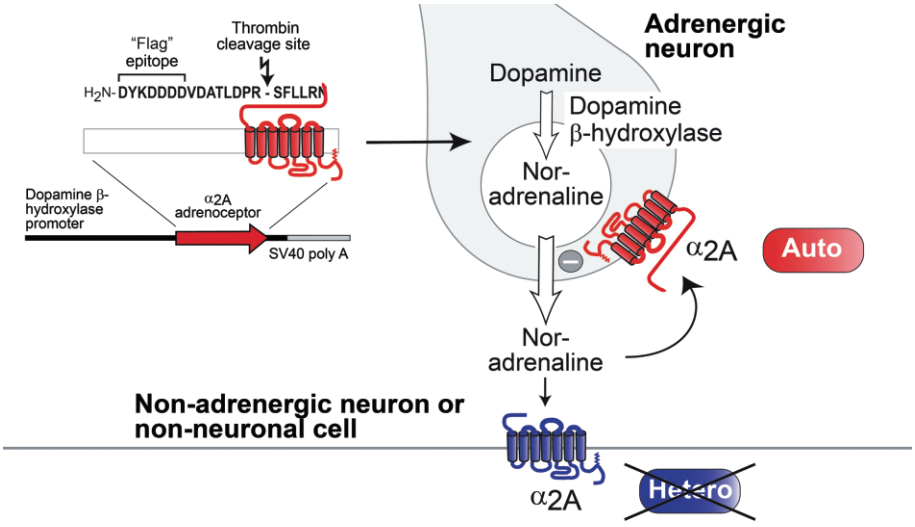

Figure 1

Transgenic model to dissect α_2-autoreceptor versus heteroceptor function. The transgenic constructs consist of the Dbh promoter sequence and coding sequence of the murine α_{2A}-adrenoceptor (Gilsbach *et al.*, 2009; 2010). By genetic engineering the sequence of a thrombin-cleavable epitope ('Flag' epitope, DYKDDDD) was introduced at the α_{2A}-adrenoceptor aminoterminus. Mice expressing this transgenic construct were crossed with mice carrying a genetic deletion of the α_{2A}- and α_{2C}-adrenoceptor genes to obtain Dbh-α_{2A} mice that express α_{2A}-autoreceptors but lack all α_{2C}-adrenoceptors and α_{2A}-heteroreceptors. Auto, α_2-autoreceptor; Hetero, α_2-heteroreceptor.

containing neurons. These results confirmed the subcellular localization of transgenic α_{2A}-autoreceptors in the hippocampus (Gilsbach *et al.*, 2009).

On the functional level, transgenic α_{2A}-autoreceptor activation inhibited voltage-gated calcium channels in sympathetic neurons and mediated feedback inhibition of noradrenaline release from cardiac sympathetic nerve terminals (Gilsbach *et al.*, 2009). Thus, the transgenic receptors fulfilled the criteria for presynaptic inhibitory autoreceptors and showed the expected expression pattern in the adrenergic system.

Cardiovascular functions of α_2-adrenoceptors

Data from a genome-wide association study in different European populations have recently linked a single nucleotide polymorphism within the 3′ untranslated region of the human α_{2A}-adrenoceptor gene *ADRA2A* with increased BP (Sober *et al.*, 2009). However, fine mapping of a partial *ADRA2A* promoter fragment in more than hundred healthy subjects did not reveal an association of common variants with plasma noradrenaline levels, BP or heart rate (Kurnik *et al.*, 2006). In addition this study identified novel variants of the *ADRA2A* gene. Two of these variants were associated with elevated noradrenaline plasma levels in healthy African-American volunteers (Kurnik *et al.*, 2006). Elevated noradrenaline levels promote the development of cardiovascular diseases. In human chronic heart failure noradrenaline levels are known to be elevated and are a predictor of mortality (Cohn *et al.*, 1984).

These observations in human subjects correlate well with data from transgenic animal models. Deletion of α_{2A}-adrenoceptors in mice leads to elevated circulating noradrenaline levels. The enhanced noradrenaline concentrations in the synaptic cleft activate postsynaptic smooth muscle and cardiac myocyte adrenoceptors and cause hypertension and tachycardia respectively (Hein *et al.*, 1999; Brede *et al.*, 2002; 2003; 2004). α_{2A}-Adrenoreceptor-deficient animals were more susceptible to develop cardiac fibrosis as well as hypertrophy and finally heart failure in response to chronic cardiac pressure overload (Brede *et al.*, 2002).

Transgenic expression of α_{2A}-autoreceptors in adrenergic neurons of $\alpha_{2A/C}$-knockout mice (Dbh-α_{2A}) lowered plasma noradrenaline levels to wild-type levels at baseline (Gilsbach *et al.*, 2010). In addition the elevated BP in $\alpha_{2A/C}$-knockout mice reverted to values of wild-type mice in Dbh-α_{2A} mice. Furthermore, the degree of cardiac hypertrophy was decreased in Dbh-α_{2A} mice. These results indicate that feedback inhibition of noradrenaline release from sympathetic nerves by α_{2A}-autoreceptors limits noradrenaline exocytosis and thus protects the heart under normal resting conditions. After chronic activation of the sympathetic system by cardiac pressure overload, the transgenic α_{2A}-autoreceptors no longer limited the noradrenaline release and the animals showed the same detrimental signs of chronic heart failure as $\alpha_{2A/C}$-knockout mice (Gilsbach *et al.*, 2010).

One explanation for the loss of the protective role of α_{2A}-autoreceptors in chronic heart failure is receptor desensitization. Desensitization of α_{2A}-autoreceptors may occur due to high concentrations of catecholamines in the synaptic cleft in chronic heart disease. After chronic left ventricular pressure overload in mice, inhibition of electrically evoked noradrenaline release by α_2-adrenoceptor agonists was

blunted (Brede *et al.*, 2002; Gilsbach *et al.*, 2010), indicating desensitization of α_{2A}-adrenoceptors. These data are supported by data from clinical trials: the effects of clonidine on noradrenaline spillover or bradycardia have been shown to be diminished in patients suffering from chronic heart failure (Lang *et al.*, 1997; Aggarwal *et al.*, 2001). Comparable results have been observed after chronic inhibition of the noradrenaline transporter by reboxetine. Chronic blockade of noradrenaline reuptake has been shown to raise noradrenaline levels in the synaptic cleft (Gainetdinov and Caron, 2003). In rats, presynaptic but not somatodendritic α_2-adrenoceptors in the hippocampus were desensitized after chronic reboxetine treatment (Parini *et al.*, 2005).

Results from two independent transgenic mouse models gave evidence for the contribution of α_{2A}-adrenoceptor endocytosis to the desensitization of α_{2A}-adrenoceptor effects (Lu *et al.*, 2009; Gilsbach *et al.*, 2010). The Dbh-α_{2A} mouse carries a cleavable flag epitope at the aminoterminus of the α_{2A}-adrenoceptor to allow for sensitive immunohistochemical detection of adrenoceptors in native tissues as illustrated in Figure 1. The flag epitope may be cleaved by thrombin from cell surface receptors but not from intracellular receptors and thus monitoring of receptor internalization is possible (Daunt *et al.*, 1997; Gilsbach *et al.*, 2010). Lu and co-workers (Lu *et al.*, 2009) developed a mouse line expressing a haemagglutinin epitope at the aminoterminus of the α_{2A}-adrenoceptor to measure receptor internalization. In both studies, α_{2A}-adrenoceptor internalization was ligand selective in *in vitro* cultivated superior cervical ganglia. Clonidine and medetomidine were more potent than guanfacine or noradrenaline respectively (Lu *et al.*, 2009; Gilsbach *et al.*, 2010). Previous reports have shown that *in vitro* internalization is not only ligand specific (Olli-Lahdesmaki *et al.*, 2004) but also influenced by the cellular environment (Olli-Lahdesmaki *et al.*, 1999). Marked agonist-dependent internalization was observed in neuronal PC12 cells as compared with non-neuronal cell lines (Olli-Lahdesmaki *et al.*, 1999).

A major mechanism for desensitization and internalization of α_2-adrenoceptors is initiated upon receptor activation by G-protein receptor kinase 2 (GRK2) (Figure 2). GRK2 binds to activated α_{2A}-adrenoceptors and phosphorylates several serine residues within the third intracellular loop (Eason *et al.*, 1995; Jewell-Motz and Liggett, 1996). In addition protein kinase C-dependent phosphorylation of a specific serine within the third intracellular loop of α_{2A}-adrenoceptors was described (Liang *et al.*, 1998; 2002). Phosphorylation of GPCRs recruits β-arrestins, which uncouple receptors from G-proteins, induce receptor endocytosis and thus receptor desensitization. However, β-arrestin binding may also initiate G-protein-independent signalling events, including activation of ERK (Rajagopal *et al.*, 2010). The endogenous protein spinophilin was shown to block GRK2 association with GPCRs (Wang *et al.*, 2004). Therefore, it can be regarded as an endogenous antagonist of GRK2 and β-arrestin signalling (Wang *et al.*, 2004). Surprisingly, spinophilin knockout mice show a gain in sensitivity to α_{2A}-adrenoceptor-mediated sedation, hypotension, bradycardia and enhanced coupling to G-proteins (Lu *et al.*, 2010). These observations cannot be attributed to augmented desensitization. They indicate that α_{2A}-adrenoceptor responses *in vivo* are at least to some degree mediated by β-arrestin-dependent signalling pathways. First evidence for this hypothesis is a loss of sensitivity to α_2-adrenoceptor agonist elicited sedation, which was observed in β-arrestin 3-deficient mice (Wang *et al.*, 2004).

In addition to noradrenaline that is released from sympathetic nerves, adrenaline, which is secreted from the adrenal medulla, is of importance for the progression of chronic heart failure. Plasma levels of adrenaline are significantly elevated in patients with terminal heart disease (Anker *et al.*, 1997) and in animal models of chronic heart failure (Lymperopoulos *et al.*, 2007; Schneider *et al.*, 2011). Since induction of GRK2 in the adrenal medulla was observed in these animals (Lymperopoulos *et al.*, 2007; Schneider *et al.*, 2011), desensitization of α_2-autoreceptors in the adrenal medulla was proposed as a mechanism contributing to elevated circulating adrenaline levels in heart failure (Lymperopoulos *et al.*, 2007; 2008; 2010). Desensitization of adrenal α_2-adrenoceptors by GRK2 may diminish α_2-autoreceptor-mediated inhibition of catecholamine secretion. Furthermore the adrenal gland increases catecholamine synthesis and undergoes hypertrophy in chronic heart disease (Lymperopoulos *et al.*, 2007) in an α_{2C}-adrenoceptor independent manner (Schneider *et al.*, 2011). Experiments on isolated chromaffin cells showed that Ca^{2+} channel activation and catecholamine release relies on α_{2C}-adrenoceptors (Brede *et al.*, 2003). Heterozygous deletion of this receptor subtype leads to elevated adrenaline secretion and the α_2-adrenoceptor agonist-mediated inhibition of catecholamine secretion was reduced to 50 %. After transverse aortic constriction heterozygous α_{2C}-knockout mice were more susceptible to develop cardiac hypertrophy and fibrosis and had a reduced survival time as compared with wild-type mice (Gilsbach *et al.*, 2007). A reason for the high correlation of α_{2C}-adrenoceptor expression and function in chromaffin cells is operation without a significant receptor reserve (Gilsbach *et al.*, 2007). By contrast, α_2-autoreceptors in sympathetic neurons have a high receptor reserve (Adler *et al.*, 1987; Agneter *et al.*, 1997). Therefore, changes in the receptor density of α_2-autoreceptors in sympathetic neurons do not correlate with functional changes as directly as changes in the density of α_{2C}-autoreceptors in chromaffin cells. Thus desensitization of α_2-adrenoceptors in adrenergic neurons and chromaffin cells may accelerate the progression of cardiovascular diseases.

It has been known for decades that α_2-adrenoceptor agonists like clonidine lower systolic and diastolic pressure as well as heart rate by acting in the CNS. In these studies clonidine was shown to inhibit the activity of sympathetic nerves and to increase the activity of parasympathetic nerves (Hoefke and Kobinger, 1966; Katic *et al.*, 1972; Laubie and Schmitt, 1977). The involvement of postsynaptic α_2-adrenoceptors in these responses was shown by an elegant combination of chemical denervation and α_2-adrenoceptor selective ligands (Kobinger, 1983). The hypotensive effect of α_2-adrenoceptor agonists was lost and the bradycardic effect was significantly attenuated in animals carrying a genetic deletion or a point mutation in the α_{2A}-adrenoceptor (MacMillan *et al.*, 1996), demonstrating the essential role of the α_{2A}-adrenoceptor in these responses. Acute α_2-adrenoceptor agonist treatment of mice expressing only transgenic α_{2A}-autoreceptors (Dbh-α_{2A}) revealed the minor role of α_{2A}-autoreceptors for the hypotensive and bradycardic effect (Figure 3, Gilsbach *et al.*, 2010). The acute bradycardic and

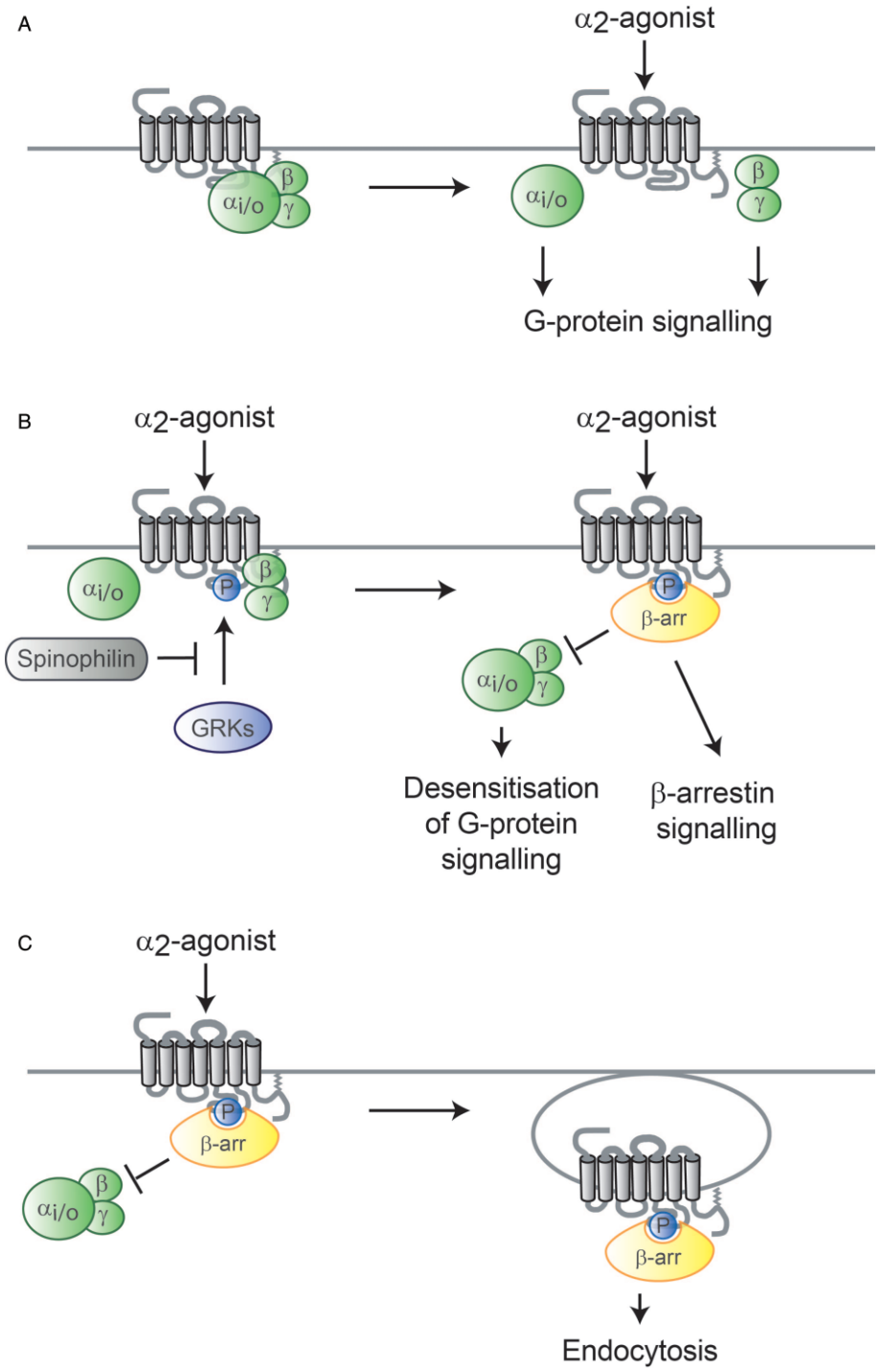

Figure 2

Schematic overview of α_2-adrenoceptor signalling, desensitization and internalization. (A) Activation of α_2-adrenoceptors induces G-protein signalling. (B) Activated α_2-adrenoceptors interact with $\beta\gamma$-subunits and G-protein coupled receptor kinases (GRKs). This complex enables GRKs to phosphorylate α_2-adrenoceptors. Spinophilin is able to block complex formation. β-arrestins (β-arr) bind to phosphorylated α_2-adrenoceptors and activate β-arrestin-dependent signalling. These processes desensitize G-protein signalling. (C) β-Arrestin binding initiates α_2-adrenoceptor endocytosis.

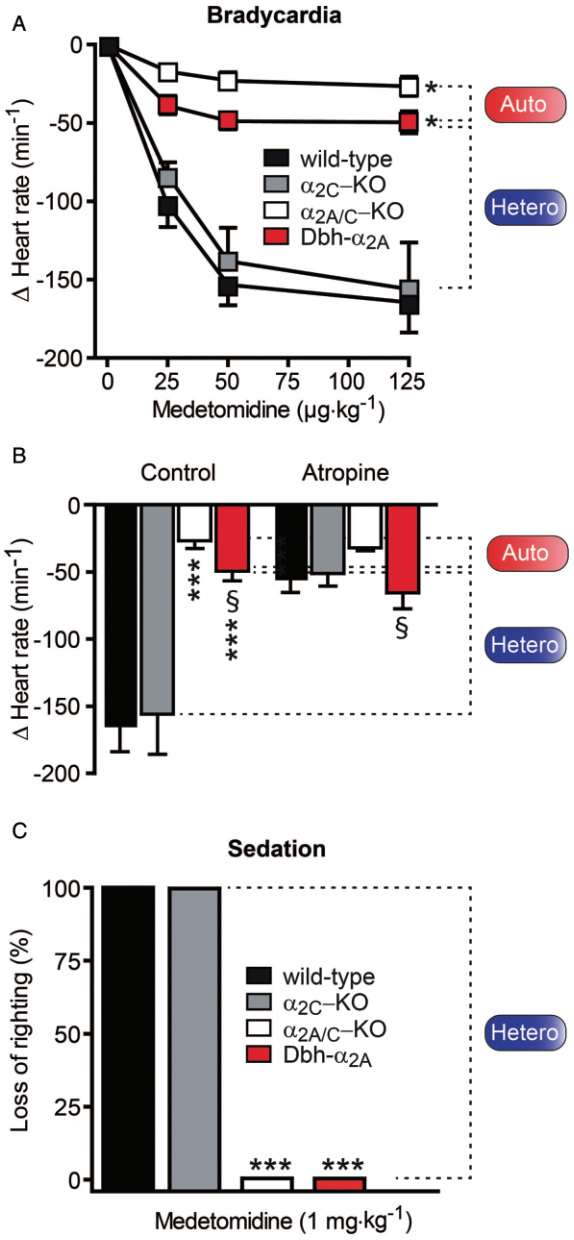

Figure 3

Bradycardic and sedative effect of the α_{2A}-adrenoceptor agonist medetomidine. Changes in heart rate (A, B) in response to intravenous infusion of medetomidine were determined by arterial microtip catheterization of mice during isoflurane anaesthesia. Shown are results from wild-type mice and mice carrying deletions of the α_{2C}-adrenoceptor gene (α_{2C}-KO) or of the α_{2A}- and α_{2C}-adrenoceptor genes ($\alpha_{2A/C}$-KO) as well as mice expressing α_{2A}-autoreceptors on a $\alpha_{2A/C}$-deficient background (Dbh-α_{2A}). (A) Cumulative effects of increasing doses of intravenous medetomidine on heart rate. (B) The maximal dose of medetomidine (125 μg·kg^{-1}) was applied in the presence or absence of atropine (modified from Gilsbach *et al.*, 2010). (C) A loss of righting reflex after application of medetomidine (1 mg·kg^{-1}) indicates deep sedation (modified from Gilsbach *et al.*, 2009). *$P < 0.05$, ***$P < 0.001$ versus wild-type; §$P < 0.05$, §§$P < 0.01$ versus $\alpha_{2A/C}$-KO. Red and blue boxes illustrate the relative contribution of α_{2A}-auto- and heteroreceptors respectively. Auto, α_2-autoreceptor; Hetero, α_2-heteroreceptor.

hypotensive effects of α_2-adrenoceptor agonists were thus primarily mediated by α_{2A}-heteroreceptors in non-adrenergic cells (Figure 3A and B). This was further supported by the finding that the α_{2A}-heteroreceptor-dependent bradycardic and hypotensive component was highly atropine-sensitive, demonstrating the involvement of vagal neurons (Figure 3, Gilsbach *et al.*, 2010). Activation of α_2-heteroreceptors dampens the effect of inhibitory GABAergic neurons projecting to cardiac vagal neurons in the *nucleus ambiguus*. Consequently the parasympathetic activity to the heart is enhanced (Philbin *et al.*, 2010), involving changes in the firing pattern of cardiac vagal motoneurons and increased sensitivity of the central limb of the baroreceptor reflex (Philbin *et al.*, 2010). In conscious mice clonidine greatly increased heart rate variability and baroreflex sensitivity and both of these effects were atropine-sensitive (Tank *et al.*, 2004b). The observations in these animal models are in good agreement with human data showing increased parasympathetic tone and increased baroreflex-mediated bradycardia after clonidine treatment (Tank *et al.*, 2004a).

The discussed studies strongly suggest that α_{2A}-autoreceptors only play a very small role in acute regulation of bradycardia, hypotension and of baroreceptor sensitivity by α_2-adrenceptor agonists. All of these effects rely mainly on α_{2A}-heteroreceptors and involve parasympathetic activation.

α_2-Adrenoceptor mediated sedation

α_2-Adrenoceptor agonists are used as sedative, hypnotic and anaesthetic-sparing agents in humans and animals (Kamibayashi and Maze, 2000; Scholz and Tonner, 2000; Sinclair, 2003; Sanders and Maze, 2007b). In mice these effects are mediated by α_{2A}-adrenoceptors (Hunter *et al.*, 1997; Lakhlani *et al.*, 1997). Remarkably, deletion of a single α_{2A}-adrenoceptor allele prevented sedation by full α_2-adrenoceptor agonists but did not affect the hypotensive action of these substances, indicating that a greater number of α_{2A}-adrenoceptors must be activated to evoke sedation than to lower BP (Tan *et al.*, 2002). Previous studies suggested that the activation of α_{2A}-autoreceptors in *locus coeruleus* neurons is essential for the sedative properties of α_2-adrenoceptor agonists (Mizobe *et al.*, 1996). Surprisingly, only in mice with functional α_{2A}-heteroreceptors, application of high doses of the agonist medetomidine induced strong sedation, indicated by the loss of righting reflex (Figure 3, Gilsbach *et al.*, 2009). This finding was supported by experiments in mice carrying a deletion in the Dbh gene, which ablates noradrenaline synthesis (Thomas *et al.*, 1995; Weinshenker and Szot, 2002). Results from these mice and from mice with chemical denervation of noradrenergic neurons (Spyraki and Fibiger, 1982; Monti *et al.*, 1988) confirmed that inhibition of noradrenaline release is not involved in the sedative effects of α_{2A}-adrenoceptor agonists *in vivo*. Interestingly, α_{2A}-autoreceptors are important for the physiological regulation of spontaneous locomotor activity at night-time (Gilsbach *et al.*, 2009). Activation of α_2-adrenoceptors has been observed to increase the sleeping time but to reduce the rapid eye movement sleep in man (Autret *et al.*, 1977). Cumulative evidence supports the involvement of endogenous sleep-promoting pathways in α_2-adrenoceptor agonist-

mediated sedation (Nelson *et al.*, 2003). α_2-Autoreceptors reduce the activity of adrenergic neurons projecting from the *locus coeruleus* to GABAergic neurons of the ventrolateral preoptic area. These neurons are disinhibited and release GABA on the *tuberomammillary nucleus*, which initiates the release of histamine on cortical neurons leading to sedation (Nelson *et al.*, 2002; 2003). Apart from the *locus coeruleus* neurons the activity of non-adrenergic neurons projecting to the ventrolateral preoptic can be modulated by α_2-adrenoceptor agonists (Matsuo *et al.*, 2003; Liu *et al.*, 2010). Furthermore, α_2-adrenoceptor agonists directly inhibit histamine release in the brain cortex (Hill and Straw, 1988; Gulat-Marnay *et al.*, 1989). Thus, α_2-autoreceptors and α_2-heteroreceptors are involved in the sleep-promoting pathway. Ablation of the G-protein α-subunit i2 ($G\alpha_{i2}$) but not of $G\alpha_{i1}$ or $G\alpha_{i3}$ attenuates the sedative effect of medetomidine and brimonidine (Albarran-Juarez *et al.*, 2009). These results indicate that coupling of either α_{2A}-adrenoceptors or other GPCRs to $G\alpha_{i2}$ is relevant for α_2-adrenoceptor agonist-mediated sedation.

Furthermore, ablation of $G\alpha_{i2}$ expression prevented the anaesthetic-sparing effect of α_2-adrenoceptor agonists (Albarran-Juarez *et al.*, 2009). This effect was also absent in $\alpha_{2A/C}$-adrenoceptor knockout mice and could not be rescued by the Dbh-α_{2A} transgene. Consequently the sedative, hypnotic and anaesthetic-sparing effects are mediated by α_{2A}-heteroreceptors and not by α_{2A}-autoreceptors in adrenergic nerves and nuclei including the *locus coeruleus*. Further studies are required to identify the involved neurons in α_{2A}-heteroreceptor-mediated sedation.

Antinociceptive effects of α_2-adrenoceptor agonists

Antinociceptive actions of α_2-adrenoceptor agonists and the involved neuronal circuits have been intensively studied (Fairbanks *et al.*, 2009). The α_{2A}-adrenoceptor seems to be the essential subtype for the antinociceptive effects of α_2-adrenoceptor agonists (Hunter *et al.*, 1997; Stone *et al.*, 1997; Fairbanks and Wilcox, 1999). However, additional receptors subtypes, for example, the α_{2C}-adrenoceptor, are likely to be involved in the antinociceptive effect of some α_2-adrenoceptor agonists, including moxonidine (Fairbanks *et al.*, 2002). Descending noradrenergic fibres project to spinal afferent neurons and control pain signals (Fairbanks *et al.*, 2009). Within the spinal cord the highest density of α_2-adrenoceptor was detected in the superficial layers of the dorsal horn (Seybold and Elde, 1984; Unnerstall *et al.*, 1984). In functional studies α_2-adrenoceptor agonists inhibited glutamatergic neurotransmission *in vitro* in primary afferent fibres of the spinal cord (Kawasaki *et al.*, 2003). *In vivo* studies suggest the involvement of presynaptic and postsynaptic sites in the antinociceptive effect of α_2-adrenoceptor agonists (Sonohata *et al.*, 2004). It is likely that the activated α_2-adrenoceptors are located in non-adrenergic neurons, since the analgesic effect of medetomidine was absent in Dbh-α_{2A} transgenic mice (Gilsbach *et al.*, 2009). After chemical denervation of noradrenergic neurons by DSP-4 the analgesic effect of α_2-adrenoceptor agonists was even increased (Post *et al.*, 1985; 1987).

α_2-Adrenoceptors modulate thermoregulation

The α_2-adrenoceptor agonists dexmedetomidine and clonidine may be used to treat postoperative shivering (Joris *et al.*, 1993; Horn *et al.*, 1997; Bicer *et al.*, 2006; Pitoni *et al.*, 2011). A human study indicates that α_2-adrenoceptor agonists attenuate the thermoregulatory control of the body by lowering the vasoconstriction and shivering threshold without affecting the sweating threshold (Talke *et al.*, 1997). Altogether these effects favour the development of hypothermia after application of α_2-adrenoceptor agonists in humans. Studies in transgenic mice indicate that α_{2A}- and α_{2C}-adrenoceptors are involved in the hypothermic effect of α_2-adrenoceptor agonists (Hunter *et al.*, 1997; Sallinen *et al.*, 1997; Lahdesmaki *et al.*, 2003; Bexis and Docherty, 2005) and that also non-shivering thermogenesis is suppressed by α_2-adrenoceptor agonists (Hocker *et al.*, 2008). The hypothermic effect of medetomidine in mice was primarily mediated by the α_{2A}-subtype (Gilsbach *et al.*, 2009). In Dbh-α_{2A} transgenic mice no effect of medetomidine on body temperature was detected, indicating the essential role of α_{2A}-heteroreceptors for the hypothermic effect (Gilsbach *et al.*, 2009). These data are supported by earlier findings in rats after application of the noradrenergic neurotoxin DSP-4 (Minor *et al.*, 1989).

Clinical relevance

The findings discussed above are relevant for the clinical application of α_2-adrenoceptor agonists. As highlighted above, α_2-adrenoceptor agonists have been successfully used for several therapeutic indications (Crassous *et al.*, 2007b; Knaus *et al.*, 2007b). During surgical or invasive diagnostic procedures, α_2-adrenoceptor agonists are used as sedatives and to lower the need for inhalative anaesthetics (Coursin and Maccioli, 2001). The type of sedation induced by α_2-adrenoceptor agonists is unique. Patients are sedated but can be aroused easily and quickly fall back into sedation again (Coursin and Maccioli, 2001). In addition these α_2-adrenoceptor agonists are used to lower the sympathetic tone during cardiac surgery (Carollo *et al.*, 2008). A general benefit is the high safety of α_2-adrenoceptor agonists in acute and intermediate treatment for up to 24 h. Compared with benzodiazepines the incidence of delirium was lower after treatment with α_2-adrenoceptor agonists (Gerlach *et al.*, 2009) and respiratory depression was also less prevalent as compared with opioids (Carollo *et al.*, 2008). Notably a meta-analysis of several trials showed that premedication with an α_2-adrenoceptor agonist prevents postoperative cardiovascular complications (Wijeysundera *et al.*, 2009) and lowers mortality (Chalikonda, 2009). The limiting side effects for these indications are the profound bradycardic and hypotensive effects.

Clinical studies have tested the hypothesis that inhibition of catecholamine release by presynaptic inhibitory α_2-autoreceptors would be beneficial in chronic heart disease. Theoretically, inhibition of catecholamine release by presynaptic α_2-autoreceptors should result in similar beneficial effects for failing heart as blockade of postsynaptic

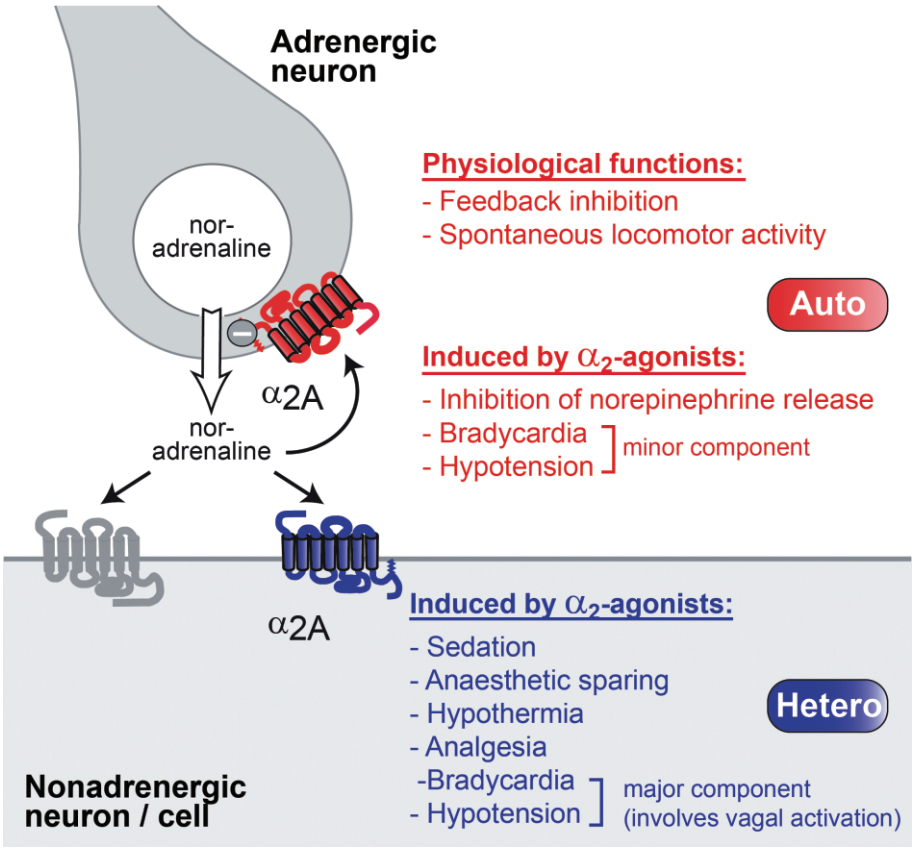

Figure 4

Schematic overview of α_2-auto- versus heteroreceptors functions. Auto, α_2-autoreceptor; Hetero, α_2-heteroreceptor; α_{2A}, α_{2A}-adrenoceptor. For references, see text.

myocardial β-adrenoceptors. In clinical trials, the α_2-adrenoceptor agonist moxonidine indeed lowered plasma catecholamines in human patients with chronic heart failure (Swedberg *et al.*, 2002). However, in the MOXCON trial (Cohn *et al.*, 2003) moxonidine caused severe side effects and even increased the mortality in chronic heart failure. The cause for the detrimental effect of moxonidine in heart failure has not been uncovered unequivocally. However, the data from experimental studies on α_2-adrenoceptors may provide a possible explanations why the clinical consequences of β-blockade may differ greatly from activation of α_2-adrenoceptors *in vivo*. On the one hand α_2-autoreceptors are likely to be desensitized in chronic heart failure due to high sympathetic tone. On the other hand application of α_2-adrenoceptor agonists may trigger several additional α_2-heteroreceptor effects. These α_2-heteroreceptor effects include augmentation of vagal tonus, which is the key factor for the hypotensive and bradycardic responses as well as enhanced baroreceptor sensitivity (Tank *et al.*, 2004b; Gilsbach *et al.*, 2010). This may explain the observed excessive bradycardic and hypotensive effect of α_2-adrenoceptor agonists as compared with β-blockers. Due to the negative outcome of the MOXCON trial (Cohn *et al.*, 2003) α_2-adrenoceptor agonists are only very restrictively used for the treatment of cardiovascular diseases. In contrast, it is suggested that α_2-adrenoceptor agonists may play a greater role in anaesthesia and critical care settings in the future (Sanders and Maze, 2007b).

Conclusions

Endogenous neurotransmitters and synthetic α_2-adrenoceptor agonists activate presynaptic α_2-autoreceptors. Under conditions of normal or acutely elevated sympathetic tone, activation of α_{2A}-autoreceptors limits noradrenaline and adrenaline spillover from sympathetic nerve endings and chromaffin cells respectively. However, the protective α_{2A}-autoreceptors are desensitized after chronic activation as it may occur in chronic heart failure. In addition, central α_{2A}-autoreceptors may be important to modulate arousal and locomotor activity (Figure 4).

The major finding of recent studies discussed in the present review is the impact of α_{2A}-heteroreceptors on non-adrenergic neurons for pharmacological effects of α_2-adrenoceptor agonists. Activation of these α_{2A}-heteroreceptors by agonists induces analgesia, hypothermia, anaesthetic-sparing, sedation/hypnosis as well as bradycardia and hypotension. This revises the current view of

α_2-adrenoceptor pharmacology in the CNS. What is the physiological role of these heteroreceptors? At present, it is unknown whether these heteroreceptors are innervated by adrenergic neurons and thus receive direct adrenergic input. Further mouse models with neuron-specific expression of α_2-adrenoceptors and advanced neurobiological experiments will be required to precisely map the cell types and signalling pathways modulated by α_2-heteroreceptors. This knowledge may pave the way to develop ligands to trigger selective effects and to identify new therapeutic targets.

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Conflict of interest

The authors have no conflict of interest related to the present work.

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