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S GUIMARÃES
Institute of Pharmacology and Therapeutics,
Faculty of Medicine
Porto, PORTUGAL

Important Recent Advances in Cardiovascular Adrenergic Mechanisms

Contents

Correspondence

Institute of Pharmacology and Therapeutics, Faculty of Medicine
Alameda Hernani Monteiro, 4200-319, Porto, Portugal

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Abstract

Five sub-areas were taken into consideration: **Adrenoceptor subclassification:** Presently, 10 different adrenoceptor subtypes have been characterized by pharmacological and molecular biology studies: α_{1A}-, α_{1B}-,α_{1D}-,$\alpha_{2A/D}$-,α_{2B}-,α_{2C}-, β_1-, β_2-, β_3- and β_4-adrenoceptors. **Intracellular signaling:** The adrenoceptors are members of a large superfamily of receptors linked to guanine-nucleotide proteins (G proteins). α_1-Adrenoceptors are coupled to G_q proteins and activate phospholipases, especially phospholipase C_β, α_2-adrenoceptors are coupled to G_i proteins and inhibit adenylyl cyclase and in some tissues regulate potassium and calcium channels. Both β_1 and β_2-adrenoceptors are preferentially coupled to adenylyl cyclase through G_s proteins and β_3-adrenoceptors appear to be coupled to K^+ channel through a pertussis toxin-sensitive G_i protein. β_4-Adrenoceptors appear to be coupled positively to a cyclic AMP-dependent cascade and can undergo desensitization. **Influence of maturation and ageing:** From birth to old age important changes occur in animal models as in humans at the receptor level, neurotransmitter process and catecholamine disposition. In general terms, one can say that maturation is associated with a gradual increase of adrenergic influence, while ageing is associated with a reduction in the role of the adrenergic system on the regulation of physiologic processes. **Cotransmission:** ATP and the neuropeptide Y are cotransmitters with noradrenaline. While noradrenaline is the main transmitter in vascular tissues, ATP has functional relevance in some vessels and neuropeptide Y is mainly a modulator of noradrenaline release; it seems that it has an increased role under pathophysiological conditions like ischemia. **Role of endothelium on noradrenaline release:** Many substances produced by the endothelium or acting through the endothelium are able to influence noradrenaline release from sympathetic nerve varicosities of the blood vessel wall: some of them, like bradykinin and angiotensin II, exert a facilitatory, while others like NO and endothelin have an inhibitory effect on noradrenaline release evoked by electrical nerve stimulation.

Key-words: vascular physiology, adrenoceptor subclassification, intracellular signaling, cotransmission, endothelium, maturation and ageing, catecholamine disposition.

Introduction

Directly or indirectly, the blood vessels are the source of many and serious diseases which affect millions of people. On the other hand, in many respects, vascular physiology and pharmacology changed dramatically over the last years. The discovery by Furchgott and Zawadzski in 1980 of EDRF (endothelium-derived relaxing factor) revolutionized our knowledge and placed the endothelium in the center of the physiology and pathophysiology of the vascular tree; the cloning of the receptors and the possibility to get transgenic animals represent not only a new and important tool for a detailed study of the adrenoceptors of the vascular system, but also a way to determine the role of each subtype of the adrenoceptor in the physiology of the vascular system. In the present review recent advances in the field of vascular adrenergic mechanisms will be summarized and highlighted. Five main areas will be dealt with: 1) adrenoceptor subclassification; 2) intracellular signaling; 3) maturation and ageing; 4) cotransmission; 5) endothelium/endocardium and transmitter release.

Some reviews covering part of the present theme were published in the last years (Insel, 1996; Starke et al., 1996; Hieble et al., 1997; Summers et al., 1997; Docherty, 1998; Miller, 1998; Bünemann et al., 1999; Freissmuth et al., 1999; Guimarães & Moura, 1999).

Adrenoceptors Subclassification

The adrenergic receptors (adrenoceptors) are the cell membrane sites through which noradrenaline and adrenaline act as important neurotransmitters and hormones in the periphery and in the central nervous system. The adrenoceptors are targets for many therapeutically important drugs, including those for some cardiovascular diseases, asthma, prostatic hypertrophy, nasal congestion and obesity.

In 1948, Ahlquist noted two patterns in the relative ability of several sympathomimetic agonists to cause pharmacological responses in a series of organs and proposed the division of adrenoceptors into two types, α and β. This was further confirmed by the identification of selective antagonists for these two sites: phentolamine and ergotamine for α-adrenoceptors; dichloroisoprenaline and propranolol for β-adrenoceptors. Nineteen years later it was shown that certain agonists and antagonists could distinguish β-adrenoceptor-mediated responses among tissues such as cardiac muscle and bronchial smooth muscle, implying the existence of subtypes of β-adrenoceptors (β_1 in cardiac muscle and β_2 in the bronchi; Lands et al., 1967). Later on, the existence and differential tissue localization of α_1 and α_2 subtypes of α-adrenoceptors were discovered and characterized. The existence of subclasses of α-adrenoceptors has become evident from the results obtained by Starke and co-workers, who showed that pre- and postjunctional α-adrenoceptors differ with respect to the relative potencies of some agonists: low concentrations of clonidine and oxymetazoline selectively activate the prejunctional α-adrenoceptors, whereas phenylephrine and methoxamine selectively activate the postjunctional α-

adrenoceptors (Starke, 1972; Starke et al., 1974, 1975[b]). Similarly, the relative potency of antagonists increased the evidence favouring this differentiation: phenoxybenzamine was about 30 times more potent in blocking postjunctional than prejunctional α-adrenoceptors (Dubocovich & Langer, 1974) and yohimbine preferentially blocked prejunctional α-adrenoceptors (Starke et al., 1975[a]). Then, Langer (1974) suggested that α-adrenoceptor-mediated responses of the effector organ should be referred to as α_1 and those mediating a reduction of the transmitter release during nerve stimulation as α_2. Later on it was verified that α-adrenoceptors pharmacologically very similar to the so-called prejunctional α_2-adrenoceptors are also found postjunctionally. In consequence the nomenclature of α_1- and α_2-adrenoceptors, depending only on the relative potencies of certain α-agonists and antagonists, was accepted from this moment on (Berthelsen & Pettinger, 1977). In the late 1980s, the development of more selective drugs and the use of molecular cloning technology showed that there are more adrenoceptor subtypes than previously suspected. Ten different subtypes have now been cloned and pharmacologically characterized (IUPHAR, 1999)

α_1-Adrenoceptors

α_1-Adrenoceptors were first divided into two subtypes α_{1A} and α_{1B} based on the differential affinity of the receptors for 5-methyl urapidil (5-MU) and WB4101 (Morrow & Creese, 1986; Gross et al., 1988; Hanft & Gross, 1989) and the irreversible antagonist chloroethylclonidine (CEC) (Han et al., 1987). α_{1A}-Adrenoceptors show high affinity for 5-MU and WB4101 and are insensitive to CEC and α_{1B}-adrenoceptors are sensitive to CEC and have low affinity for 5-MU and WB4101. The true "Renaissance" in receptor pharmacology (Kenakin, 1997), which began ten years ago with the cloning of the first receptor, had also profound influence in the study of adrenoceptors: three recombinant α_1-adrenoceptor proteins were identified and three native α_1-adrenoceptors have been characterized pharmacologically. Unfortunately, the relationships between the native and the recombinant α_1-adrenoceptor subtypes and the nomenclature used to identify them led to some confusion. The α_{1b}-adrenoceptor subtype (lower case subscripts are used to denote the recombinant α_1-adrenoceptor subtypes, and the upper case subscripts refer to the native α_1-adrenoceptors found in tissues or cells) was the first to be cloned from the hamster (Cotecchia et al., 1988) and this clone expressed a protein showing binding properties identical to those of the α_{1B}-adrenoceptor. The next clones were the bovine α_{1c} (Schwinn et al., 1990), the rat α_{1a} (Lomasney et al., 1991) and the rat α_{1d} (Perez et al., 1991). However, the α_{1a} and α_{1d} recombinant adrenoceptor proteins showed 99.8% homogeneity and appeared to represent the same subtype. Accordingly, at present a consensus statement has been obtained, such that the subdivision in three subtypes is widely accepted: α_{1A} (α_{1a} for the recombinant adrenoceptor), α_{1B} (or α_{1b}) and α_{1D} (or α_{1d}) (Ford et al. 1994; Bylund et al., 1994). In humans, α_{1A}, α_{1B}, and α_{1D}-adrenoceptors are encoded by distinct genes located on chromosomes 8, 5 and 20, respectively (Michel et al., 1995). A fourth α_1-adrenoceptor, the so-called α_{1L}-adrenoceptor has been postulated (Holck et al., 1983; Flavahan & Vanhoutte, 1986), based exclusively on pharmacological criteria (e.g., relatively

low affinity for prazosin) yet the cDNA for it has not been isolated. Very recent studies provide evidence that the α_{1L}-adrenoceptor may not derive from a distinct gene, but represent a particular, energetically favourable conformational state of the α_{1A}-adrenoceptor (Ford et al.,1998). Some discrepancies which have been observed when a comparison is made of results obtained by functional, radioligand binding and molecular biology studies must be carefully examined . For example, the two most selective agents currently used in the characterization of α_1-adrenoceptor-subtypes are 5-MU and (+)-niguldipine which are 50- to 100-fold selective for α_{1A} over the α_{1B}-subtypes. However, 5-MU is also a potent 5-hydroxytryptamine receptor agonist (Gross et al., 1989) and (+)-niguldipine is a potent calcium channel blocker (Boer et al.,1989); either of these two effects can drastically affect the results obtained in functional studies without interfering in radioligand binding studies.

The α_1-adrenoceptors are the most thoroughly characterized of the α-adrenoceptor family and vasoconstriction is one of the most prominent functions mediated by α_1-adrenoceptors. However, other functions with pathophysiological implications are important enough to justify the interest in developing subtype-specific drugs that are more effective and have fewer side effects than those now available. For example, Halotano et al. (1994) reported a slightly lower potency for 5-MU and WB4101 in the human iliac artery compared to the human urethra and this yielded the hypothesis that α_1-selective drugs might be therapeutically effective in the symptomatic treatment of benign prostatic adenoma without causing the vascular side effects associated with α_1-adrenoceptor blockade. In a recent direct comparison between tetrazosin and tamsulosin in doses equally effective in patients with prostatic hyperplasia, it was observed that tamsulosin caused significantly fewer side effects (Lee & Lee, 1997). Unfortunately, further experimental and clinical data do not unequivocally support this concept (Schäffers et al., 1998). However, some new drugs which are more selective for α_{1A}-adrenoceptor are now in clinical development.

α_2-Adrenoceptors

It is now clear that there are three subtypes of α_2-adrenoceptors: $\alpha_{2A/D}$-, α_{2B}- and α_{2C}. This subdivision although primarily based on radioligand binding data, was preceded by results obtained by functional and was confirmed by molecular cloning studies. The α_{2B}- and α_{2C}-adrenoceptors have similar pharmacological characteristics between species; however, the α_{2a}-adrenoceptor cloned from human and porcine tissue differs slightly in its amino acid composition from the homologous receptor cloned from rat, mouse or guinea pig in having a serine residue rather than a cysteine, at the position corresponding to Cys^{201} in the α_{2a}-adrenoceptor. To three different genes correspond four pharmacological subtypes since the same gene expresses as α_{2a} in the dog, rabbit, man and as α_{2D} in the mouse, rat and guinea pig (Bylund et al., 1994; Starke et al., 1995; Trendelenburg et al., 1996; Paiva et al., 1997); Guimarães et al., 1998[a]). In humans, the genes expressing α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors are localized in chromosome 10, 2 and 4, respectively (Regan et al., 1988; Lomasney et al., 1990; Weinshank et al., 1990).

Pharmacologically it is well known that the different α-adrenoceptor antagonists possess different potency / affinity for the different α_2-adrenoceptor subtypes: prazosin for example, has high affinity for α_{2B}- and for α_{2C}-adrenoceptors and very low affinity for α_{2A}- and for α_{2D}-adrenoceptors (Latifpour et al., 1982; Bylund et al., 1988; Nahorski et al., 1985); on the other hand, yohimbine and rauwolscine are more potent than phentolamine and idazoxan on α_{2A}-adrenoceptors, whereas phentolamine and idazoxan are more potent than yohimbine and rauwolscine on α_{2D}-adrenoceptors (Starke, 1981; Ennis, 1985; Lattimer & Rhodes, 1985; Alabaster et al., 1986; Limberger et al., 1989). The comparison of the functional potency of several antagonists with their affinity to all subtypes, as determined either in radioligand assays to native tissues possessing only one subtype or to express recombinant α_2-adrenoceptors shows a full agreement. So, this functional approach has been extensively used to characterize α_2-autoreceptor subtypes in the different tissues (Hieble et al., 1997). Systematic studies recently undertaken to characterize prejunctional α_2-adrenoceptor subtypes in different species confirmed that receptors with α_{2A} properties occur in humans, pigs and rabbits, while receptors with α_{2D} properties occur in rats, mice and guinea pigs (Bylund et al., 1994; Starke et al., 1995; Trendelenburg et al., 1996; Paiva et al., 1997; Guimarães et al., 1998[a]). However, some rare discrepancies to this premise have been reported by different authors: in the rat vena cava (Molderings & Göthert, 1993) and rat atria (Connaughton & Docherty, 1990) where the prejunctional receptors were classified as α_{2B} and in human kidney cortex (Trendelenburg et al., 1994) and human right atrium (Rump et al., 1995) where they appeared to belong to α_{2C}-subtype. However, a re-investigation of these unexpected subclassifications showed that the prejunctional receptors in rat vena cava and atria and in guinea-pig urethra were α_{2D} and those of human kidney were α_{2A}. Thus, in contrast to previous suggestions, all these receptors confirm to the rule that α_2-autoreceptors belong, at least predominantly to the genetic $\alpha_{2A/D}$-subtype (Trendelenburg et al., 1997).

Transgenic mice lacking only one of the α_2-adrenoceptor genes were used to elucidate the physiological function of each α_2-adrenoceptor subtype *in vivo*. The results show that central sympathetic outflow is primarily regulated by α_{2A}-adrenoceptors, and that the central hypotensive response to α_2-adrenoceptor-agonists is not mediated by either the α_{2B}- or the α_{2C}-subtypes (MacMillan et al., 1996). The major component of the α_2-adrenoceptor agonist-induced increase in systemic blood pressure, on the other hand, is mediated by α_{2B}-adrenoceptors of the vascular smooth muscle (MacMillan et al., 1996; Hein et al., 1998).

Prejunctionally, it was observed that maximal inhibition by α_2-agonists of the electrically-evoked contractions of the vas deferens was reduced to 50% in mice lacking α_{2A}-adrenoceptors, whereas the effect of these agonists in α_{2C}-adrenoceptor-deficient mice was unchanged. However, in mice lacking both the α_{2A}- and the α_{2C}-adrenoceptors the prejunctional effect of α_2-agonist was abolished, indicating that the residual response (50%) to α_2-agonists in α_{2A}-knockout animals was due to presynaptic α_{2C}-adrenoceptors (Hein et al., 1998). Thus, the possibility that more than one α_2-adrenoceptor subtype may be present at prejunctional level in the same tissue (Guimarães et al., 1997; Ho et al., 1998; Docherty, 1998) seems to be confirmed.

The vast majority of studies aiming at subclassifying α_2-adrenoceptors dealt with prejunctional autoreceptors.

On the basis of functional studies, it was reported that postjunctional α_2-adrenoceptors of the human saphenous vein belong to α_{2A}-subtype (Hicks et al., 1991; MacLennan et al., 1997). In agreement with that premise, it was observed that postjunctional α_2-adrenoceptors of the canine mesenteric vein are α_{2A} while those of the rat femoral vein are a_{2D} (Paiva et al., 1999). However, Gavin et al. (1997) showed that postjunctional α_2-adrenoceptors of the human saphenous vein are α_{2C}.

Although the vast majority of tissues express more than one subtype, there are rare tissues expressing only one subtype: α_{2A} in human platelets (Bylund et al., 1988), α_{2B} in rat neonatal lung (Bylund et al., 1988), α_{2C} in opossum cells (Murphy & Bylund, 1988) and α_{2D} in the rat submaxillary gland (Michell et al., 1989)

β-Adrenoceptors

The classical β_1- and β_2-adrenoceptors mediate responses to noradrenaline released from sympathomimetic nerve terminals and to circulating adrenaline. They are stimulated or blocked by many compounds which produce valuable effects in the treatment of important and common diseases like hypertension, cardiac arrhythmias, ischaemic heart disease and asthma. On the basis of many pharmacological and molecular studies, there is now good evidence for the existence of four beta adrenoceptor subtypes (β_1, β_2, β_3 and β_4; for reviews, see Arch & Kaumann, 1993; Barnes, 1995; Strosberg & Pietri-Rouxel, 1996; Strosberg, 1997; Summers et al., 1997). β_1- and β_2-adrenoceptors are well known since the classical paper by Lands et al. (1967). An additional subtype, the β_3-adrenoceptor was first suggested by the observation that lipolysis in response to sympathomimetic stimulation was resistant to blockade by propranolol (Harms et al., 1974). Atypical β-adrenoceptor-mediated responses obtained in white and brown fat and in the gastrointestinal tract which are not blocked by propranolol and other conventional β-adrenoceptor antagonists and are stimulated by novel lipolytic agonists are now known to be mediated by β_3-adrenoceptors (for reviews see Manara et al., 1995; Summers et al., 1997). There are still no highly selective antagonists of β_3-adrenoceptors; however, a series of aryloxypropanolamínotetralins (e.g. SR 59230A) have been described that are at least 30-fold more potent as relaxants of rat colon (β_3) than as stimulants of the atria (β_1) or relaxants of trachea (β_2). In addition to these well established receptors, other receptors have been described, for which the evidence is ambiguous or incomplete. These receptors include the receptor in rat soleus muscle, which mediates glucose uptake; the receptor in human and rat heart, which mediates positive chronotropism and inotropism; and a receptor cloned from turkey (Chen et al., 1994), which has been termed the β_4-adrenoceptor.

In 1996, Kaumann and Molenaar observed that micromolar concentrations of selective β_3-adrenoceptor agonists failed to cause cardiac stimulation; furthermore, the selective blockade of the colonic putative β_3-adrenoceptor compared to the third cardiac β-adrenoceptor by SR 59230A, as well as the blockade of cardiac but not colonic receptors by CGP 20712A was also inconsistent with an identical putative β_3-adrenoceptor in colon and heart. Although there is some evidence supporting the existence of four β-adrenoceptor populations in human heart, three identical to the recombinant β_1- β_2- and β_3-, and a fourth not yet cloned, the situation is still somewhat complex (Arch, 1998). According to this author, although the pharmacology of β_3-adrenoceptors is clearly distinct from that of β_1- and β_2-adrenoceptors, one cannot forget that there are differences between rodents and man and this also contributes to some confusion in the subclassification of β-adrenoceptors (Arch, 1998).

β_4-Adrenoceptor has been cloned from a turkey genomic library as mentioned above. This receptor has 50-60% identity with other β-adrenoceptors and like the mammalian β_3-adrenoceptors has an intron towards the 3′ end of the coding sequence. In mouse L-cells stably expressing the β_4-adrenoceptor, isoprenaline, noradrenaline and adrenaline stimulate cyclic AMP (cAMP) formation. The receptor is expressed in lung, blood, intestine, stomach, heart and brain (Chen et al., 1994). There have been no reports to date of mammalian homologues of this receptor and, based on its nucleotide sequence similarity or pharmacological properties, it does not appear to be the turkey equivalent of the β_3-adrenoceptor.

Intracellular Events Triggered by Receptor Activation

The adrenoceptors are members of a large superfamily of receptors linked to guanine-nucleotide proteins (G-proteins). The superfamily of G-protein-coupled receptors include not only the adrenoceptors but also the receptors for acetylcholine, dopamine, histamine, prostaglandins, angiotensin, oxytocin, glucagon, adenosin, etc. Each type of G-protein can be used for signalling by more than one type of receptor. For example, many different types of receptors that stimulate adenylyl cyclase activity activate G_s-proteins. The basic unit of G-protein-coupled receptor signalling consists of three parts: a heptahelical receptor that detects ligands in the extracellular medium, a G-protein that dissociates into a subunits bound to guanosine triphosphate and bg subunits after interaction with the ligand-bound receptor, and an effector that interacts with dissociated G-protein subunits to generate small-molecule second messengers. The receptor-G-protein interaction is catalytic; that is, one receptor sequentially activates multiple G-proteins (Luttrel et al., 1999).

α_1-Adrenoceptors initiate signals in their target cells by increasing the concentration of free cytosolic Ca^{2+} and thereby affecting the metabolic or contractile state of the cell. It is known that these receptors, which are coupled to a G_q-protein and activate phospholipases (especially phospholipase C_b; for a review see Insel, 1996), control cytosolic

Ca^{2+} primarily by stimulating hydrolysis of phosphatidylinositol biphosphate producing inositol triphosphate (IP_3) and diacylglycerol (DAG); while it has been shown that IP_3 releases Ca^{2+} sequestrated in intracellular nonmitochondrial pools, particularly the endoplasmic reticulum, by interacting with specific receptors, DAG can activate protein kinase C (PKC) which then phosphorylates many cellular proteins that may regulate intracellular calcium concentration (Nishizuka, 1984). Evidence is also available indicating that activation of α_1-adrenoceptors can increase influx of extracellular Ca^{2+} via voltage-dependent (Ljung & Kjellstedt, 1987) and non-voltage-dependent Ca^{2+} channels (Han et al., 1992). According to earlier suggestions α_{1A}- and α_{1B}-adrenoceptors might increase Ca^{2+} levels by different mechanisms: α_{1A}-adrenoceptors would promote influx of extracellular Ca^{2+} through dihydropyridine-sensitive channels, while α_{1B}-adrenoceptors initiate signals through the well-characterized inositol phospholipid pathway (Minneman, 1988). However, it is now generally agreed that α_{1B}-adrenoceptor activation increases formation of IP_3 and DAG and that IP_3 releases Ca^{2+} from intracellular pools. The signalling mechanism activated by α_{1A}-adrenoceptor stimulation is not yet totally clarified. In conclusion, at the moment, no close relationship can be established between specific subtypes and signalling mechanisms. While α_1-adrenoceptors are coupled to a G_q-protein and activate phospholipases, especially phospholipase C_β, α_2-adrenoceptors are coupled to a G_i-protein and inhibit adenylyl cyclase and in some tissues regulate potassium and calcium channels. From the linkage to adenylyl cyclase results a decrease in intracellular cAMP.

Both β_1- and β_2-adrenoceptors are preferentially coupled to adenylyl cyclase through a G_i-protein and β_3-adrenoceptors appear to be coupled to K^+ through a pertussis toxin-sensitive G_i-protein. Putative "β_4"-adrenoceptors appear to be coupled positively to a cAMP-dependent cascade and can undergo desensitization (Kaumann & Molenaar, 1997).

Agonist-occupied receptors couple to G-proteins which exchanges a molecule of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) and then dissociates into G_α-GTP and G_β (Northup et al., 1983). In the case of G_s, the activated $G_{s\alpha}$-GTP activates adenylyl cyclase, which leads to increased levels of the intracellular second messenger cAMP (Birnbaumer, 1992). This in turn activates protein kinase A which mediates phosphorylation of target proteins such as Ca^{2+} channels in the heart leading to metabolic and physiological responses in cells.

All of the β-adrenoceptor subtypes increase intracellular cAMP levels by coupling to Gs and activating adenylyl cyclase. This mechanism underlies the classical responses to β_1-adrenoceptor stimulation such as increases in rate and force of the heart and release of renin from the kidney juxtaglomerular apparatus; β_2- adrenoceptor stimulation such as relaxation of blood vessels and bronchial smooth muscle and β_3-adrenoceptor stimulation such as lipolysis and thermogenesis. However, some recent studies indicate that under certain circumstances, β-adrenoceptor, and particularly β_3-adrenoceptor, can couple to G_i- as well as to G_s-proteins (Asano et al., 1984; Chaudry et al., 1994; Xiao et al., 1995; Gauthier et al., 1996).

Intracellular events following β-adrenoceptor activation are also linked to ion transport. It is well known, for example that β-adrenoceptors mediate increases in cAMP , activate PKA which phosphorylates L-type Ca^{2+} channels, facilitating Ca^{2+} entry and producing the positive inotropic effect in atria and ventricles, increased heart rate in sino-auricular node and acceleration of conduction in the auriculo-ventricular node. In addition to mechanisms which indirectly lead to alterations in ion transport, β-adrenoceptor activation is more directly linked to ion channels. β-adrenoceptor stimulation is able to activate L-type Ca^{2+} channels via $G_{\alpha s}$ (Brown, 1990). In airway smooth muscle, β-adrenoceptor activation open Ca^{2+} dependent K^+ channels and charybdotoxin antagonizes the relaxant effects of β-adrenoceptor agonists (Miura et al.,1992; Jones et al., 1993).

Multiple mechanisms exist to control the signaling and density of G-protein-coupled receptors. The termination of G protein-coupled receptor signals involves binding of proteins to the receptor. This process is initiated by serine-threonine phosphorylation of agonist-occupied receptors, both by members of the G-protein-coupled receptor kinase family and by second-messenger-activated protein kinases such as adenosine 3′,5′-monophosphate-dependent kinase and PKC. Receptor phosphorylation by G-protein-coupled receptor kinase is followed by binding of proteins termed arrestins, which bind the phosphorylated receptor and sterically inhibit further G-protein activation (Luttrell et al., 1999). Desensitized receptor-arrestin complexes undergo arrestin-dependent targeting for sequestration through clathrin-coated pits (Goodman et al., 1996; Luttrell et al., 1999). Sequestrated receptors are ultimately either dephosphorylated and recycled to the cell surface or targeted for degradation (Luttrell et al., 1999).

In addition, many G-protein-coupled receptors are sequestrated from the cell membrane and become inaccessible to their activating ligands. Both receptor/G-protein uncoupling and receptor sequestration may involve the participation of arrestins or other proteins. A model for receptor regulation has been developed on the basis of data from studies of the β-adrenoceptors. However, according to recent reports other G-protein-coupled receptors, like muscarinic receptors in the cardiovascular system may be regulated by mechanisms other than those that regulate the β-adrenoceptors (for a review see Bünemann et al., 1999).

Influence of Maturation and Ageing

Maturation and ageing are associated with many alterations in vascular adrenergic mechanisms. From birth to adulthood (maturation) and from adulthood to old age (ageing or senescence) important changes occur in animal models as in humans at the receptor level, neurotransmitter process and catecholamine inactivation.

In general terms, one can accept that maturation is associated with an increase while ageing is associated with a reduction in the adrenergic influence on the physiological processes.

α-Adrenoceptors

Both α_1-adrenoceptor-mediated postjunctional effects of phenylephrine and α_2-adrenoceptor-mediated negative modulation of noradrenaline release are fully developed at birth (Guimarães et al., 1991). However, while at the postjunctional level phenylephrine is equipotent in adults and neonates, indicating that postjunctional α_1-adrenoceptors do not change during maturation, UK-14,304 is about 4 times more potent at inhibiting noradrenaline release evoked by electrical stimulation in adults than in neonates (Guimarães et al.,1991). One possible explanation for this difference is that the fractional release of noradrenaline is much higher in neonates than in adults (Guimarães et al., 1991; Moura et al., 1993). Hence, the concentration of noradrenaline in the biophase during electrical stimulation is higher in neonates than in adults; consequently, the inhibitory effect of a given concentration of UK-14,304 is smaller and its IC_{50} is higher in neonates than in adults (Starke, 1972; Fuder et al.,1983). Based on the temporal and regional pattern of α_2-adrenoceptor mRNA expression in rat brain it has been suggested that the perinatal increase in receptor density may serve specific roles in development, including neuronal migration, maturation of neurons, and mediation of sensory functions (Winzer-Serhan & Leslie, 1997; Winzer-Serhan et al., 1997[a,b]). According to Happe et al., (1999), α_2-adrenoceptors are functionally coupled to G-protein throughout postnatal development and, therefore, are able to mediate signal transduction upon stimulation by noradrenaline and adrenaline.

It is well documented that the responsiveness of vascular smooth muscle to α_1-adrenoceptor activation changes with age, although in the majority of functional studies no important changes in responses to noradrenaline had been demonstrated either during maturation or ageing (for a review see Docherty, 1990). In the dog mesenteric artery and rat aorta small reductions in the responsiveness to sympathomimetic amines were reported during maturation (McAdams &Waterfall, 1986; Toda & Shimizu, 1987), while a decrease in α-adrenoceptor-mediated functions with ageing was observed in the rat tail artery (Fouda & Atkinson, 1986) and in rat aorta (Hyland et al., 1987; Wanstall & O'Donnell, 1989). According to Satoh et al., (1995) in the rat aorta the potency of noradrenaline increased with age from 3 to 10 weeks, but decreased from 10 to 40 weeks.

It has been suggested that the age-related changes in α_1-adrenoceptor-mediated vasoconstrictor responses in isolated blood vessels might result from changes in the expression of the α_1-adrenoceptor subtypes; accordingly, functional, radioligand binding and molecular biology studies using rat aortic tissue have shown that with age the expression of the α_{1A} subtype is increased, that of the α_{1B} subtype is decreased and that of α_{1D} subtype does not change (Gurdal et al.,1995 [a,b]). However, on the basis of pressor responses caused by phenylephrine in pithed rats, Ibarra et al. (1997)

showed that in the resistance vessels of the rat, the functional expression of α_{1D}-adrenoceptors increases with age while that of the α_{1A}-adrenoceptor subtype predominates in young animals. This apparent contradiction may well be due to the fact that the aorta and the resistance vessels are functionally totally different . The results obtained by Xu et al. (1997) confirm that ageing changes heterogeneously the expression of α_1-adrenoceptor subtypes. These authors determined the changes in mRNA levels of α_1-adrenoceptor subtypes during maturation and ageing in aortae and in renal, pulmonary and mesenteric arteries isolated from 3, 12 and 24-months old rats and observed that in aorta α_{1A}-, α_{1B}- and α_{1D}- adrenoceptors declined with ageing, while in the renal artery there was a decrease in mRNA for the α_{1B}-adrenoceptor in aged rats. However, in mesenteric and pulmonary arteries there was no change in mRNA levels for any of the subtypes. The results obtained on the aggregatory responses of human platelets in radioligand binding studies also show no important differences with maturation and ageing in the affinity of ligands for the binding site (Buckley et al., 1986: Davis & Silski, 1987).

β-Adrenoceptors

In the canine saphenous vein there are no β-adrenoceptor-mediated effects at birth both at the pre- and the postjunctional level. The clear responses to forskolin, a direct acting stimulant that bypasses the need for β-adrenoceptors on their linkage to stimulatory G-protein subunits, show that the lack of responses to isoprenaline is linked to either a lack or some kind of immaturity of the receptors or of the G-protein (Guimarães et al., 1994). Furthermore, it was shown that β_2-adrenoceptor-mediated effects and the increase in the adrenaline content of the adrenal gland have a parallel time course (Paiva et al., 1994). Thus, both the prejunctional and the postjunctional β_2-adrenoceptor-mediated effects increase with increasing age (until adulthood) as does the adrenaline content of the adrenal gland, such that at two weeks β_2-adrenoceptor-mediated maximal effect is about 50% of that of the adult and at one month it is fully developed (Paiva et al., 1994). The relationship between the content of adrenaline of the adrenal medulla and the development of β_2-adrenoceptor-mediated responses was analysed also in the rat, a species in which β_2-adrenoceptor-mediated responses develop earlier than in the dog, such that at birth these responses are already fully expressed. Interestingly, while the adrenaline content of the canine adrenal medulla at birth is about 3% that of the adult, in the rat it is about 50%. This suggests a link between adrenaline and the maturation of β_2-adrenoceptor-mediated effects indicating that either adrenaline triggers the expression of β_2-mediated effects or that the expression of adrenaline formation and β_2-effects are simultaneously evoked by the same event (Moura et al., 1997).

β-Adrenoceptor-mediated relaxation was compared in the pulmonary vein of the fetal (145±2 days of gestation) and newborn lamb. Isoprenaline caused greater relaxation in newborn than in fetal lambs. Biochemical studies showed that isoprenaline

and forskolin evoked a greater increase in cAMP content and in adenyl cyclase activity of pulmonary veins in the newborn than in the fetal lamb. These results show that β-adrenoceptor-mediated relaxation of the pulmonary veins increases with maturation (Gao et al., 1998). However, according to Conlon et al. (1995), there is no change in myocardial ventricle β-adrenoceptor-G-protein coupling capacity or adenylate activation with ageing beyond maturity. These authors showed that ageing between 6 and 26 months in male Wistar rats is not accompanied by changes in myocardial β-adrenoceptor signal transduction and capacity for formation of the high affinity β-adrenoceptor-G-protein coupled complex with the agonist. It was also found that an age-related impairment of myocardial β-adrenoceptor upregulation occurs with ageing (Conlon et al., 1995).

This β-adrenoceptor-mediated relaxing capacity, which increases during the first weeks of life, then declines as the age increases. The loss of vasodilator response to isoprenaline in the rat aorta has been reported at different ages ranging from 3 to 22 months (for a review see Docherty, 1990). There is not only a decrease in the maximum relaxation to isoprenaline with ageing, which has been reported for the rabbit aorta, rat pulmonary artery, rat mesenteric artery, human saphenous vein, canine mesenteric artery, but also an increase in the $EC_{50\%}$ of isoprenaline: in the aorta of 5 and 20 weeks old rats pre-constricted with phenylephrine, the pD_2 values for isoprenaline were 7.97 and 6.57, respectively (Borkowski et al., 1992) indicating a marked reduction in the potency of this β-adrenoceptor agonist. Also SHRs exhibit an age-related loss in vasodilator β-adrenoceptor responsiveness. However, the maximum relaxation to sodium nitrite is not reduced (O'Donnell & Wanstall, 1986).

Because most reports find no change with age in the number of β_1- or β_2-adrenoceptor- binding sites of the human lymphocytes and rat heart and because cAMP production in response to forskolin and dibutyril cyclic adenosine monophosphate are also reduced by ageing in the rat myocardium and human lymphocytes, it seems likely that the change is not at receptor level but in the coupling to the adenylyl cyclase via G-proteins. In healthy volunteers of different ages, isoprenaline-induced increases in heart rate were significantly greater in young than in old ones (Brodde et al., 1998). However, β-adrenoceptor number and subtype distribution were unchanged as determined in patients undergoing open heart surgery. The decrease in β-adrenoceptor-mediated efficiency is due to a reduced activity of the catalytic unit of the adenylyl cyclase (Brodde & Pönicke, 1998).

Prolonged or repeated exposure to β-agonists in adults may result in a compensatory desensitization that reduces responsiveness (for a review, see Summers et al., 1997). In older animals, the predominant effect is heterologous desensitization mediated at the level of the G-protein. During development, however, responses in most systems increase with age and with the maturation of neuronal inputs (Giannuzzi et al.,1995). Instead of producing desensitization of responses, agonist exposure promotes receptor signaling by enhancing expression and/or catalytic efficiency of adenylyl cyclase. These developmental differences are likely to be important in the maintenance of tissue responsiveness during the period in which innervation develops (Guimarães et al., 1994; Giannuzzi et al., 1995; Moura et al., 1997).

Catecholamine Disposition

The life span of noradrenaline released into extracellular space is limited preferentially by uptake into the sympathetic nerve terminals followed by accumulation in the vesicles or deamination, while adrenaline is preferentially taken up into extraneuronal cells and degradated by O-methylation (for reviews see: Paiva & Guimarães, 1978; Osswald & Guimarães, 1983; Trendelenburg, 1988). In the liver the metabolic degradation of noradrenaline or adrenaline occurs mainly extraneuronally (Steinberg et al.,1988; Martel et al.,1993*). In vivo*, it was also shown in adult animals, that noradrenaline is preferentially subject to neuronal, but adrenaline subject to extraneuronal uptake and metabolism (Eisenhofer, 1994; Eisenhofer & Finberg, 1994).

Transport of catecholamines through the cell membranes is mediated by substrate-specific, sodium-dependent high-affinity carriers in the plasma membrane of the releasing neurons (Schloss et al., 1992) and by less specific sodium-independent, low-affinity, high-capacity extraneuronal carriers (Trendelenburg, 1988). Recently, several organic cation carriers that are members of a family of carriers termed OCT family have been cloned from various species (Gründemann et al., 1994; Okuda et al., 1996; Gründemann et al., 1997; Zhang et al., 1997). Overlap in the sensitivity to antagonists between extraneuronal catecholamine uptake and apical renal transport of organic cations by OCT_2 (Gründemann et al., 1997) led to the conclusion that the extraneuronal carrier, which was also cloned by Gründemann et al. (1998), and OCT_3 belong to the same superfamily of carriers.

The presence of an active neuronal uptake at birth was previously reported for the carotid artery of the lamb (Su et al., 1977), for the rabbit aorta (Guimarães et al., 1991) and for the canine saphenous vein (Moura et al., 1993). However, the presence of neuronal uptake at birth does not necessarily mean maturity of the sympathetic nerves since the development of the neuronal uptake precedes effective adrenergic transmission (Su et al., 1977). In contrast to the capacity of the sympathetic nerve terminals in accumulating noradrenaline, the metabolic pathways for its degradation is still immature at birth. In all tissues of the rat, the amount of metabolites formed from both MAO and COMT activities is much smaller in newborns than in adults (Guimarães et al., 1998).

In adults and old rats, the predominant metabolites formed from noradrenaline in the heart are DOPEG and DOMA, while in newborns DOMA is practically not formed. The lack of DOMA formation at a moment at which DOPEG is the metabolite largely predominant favours the view that, at birth, there is a failure of the dehydrogenating process, which can be due either to a lack of aldehyde dehydrogenase, to a lack of its co-factor or to a lack of both the enzyme and the co-factor (Guimarães et al., 1998).

With ageing (old vs. adult animals) there is a marked decline (by about 60%) in the capacity of the aorta to metabolize noradrenaline while in the heart, the capacity to degradate the same amine is markedly enhanced. Most probably, the reduction in the metabolic capacity of the aorta is linked to degenerative changes having occurred

in the vessel wall dependent on age, while the enhancement of this capacity in the heart may be related to the high noradrenaline content of the heart of old rats which are candidates for or already suffering from heart failure (Guimarães et al., 1998).

Cotransmission in Blood Vessels

Since a long time ago it is known that ATP and catecholamines coexist in both adrenal medullary chromaffin cells (Hillarp et al., 1955; Blaschko et al., 1956) and in sympathetic nerves (Schümann, 1958; Stjärne & Lishajko, 1966). The first demonstration that ATP might be released with noradrenaline from sympathetic nerves was made by Su et al. (1971). It it now widely accepted that at many sympathetic neuroeffector junctions ATP is coreleased with noradrenaline and, in a manner similar to noradrenaline, ATP exerts both postjunctional effects and prejunctional modulatory effects on transmitter release (Burnstock, 1990; Westfall et al., 1991). Cotransmission has been shown in a number of different blood vessels including rat tail artery, rabbit ear artery, canine basilar artery, mesenteric artery, rabbit pulmonary artery, guinea-pig and rabbit saphenous arteries and in rabbit hepatic artery (for a review see Burnstock, 1990).

The proportion of the cotransmitters noradrenaline and ATP varies between different vessels. For example, ATP is the major component of sympathetic cotransmission in rabbit saphenous artery and an important component in rabbit mesenteric artery but it is a relatively minor component in rabbit ear and rat tail arteries.

According to Burnstock (1990), synergism appears to be a characteristic of cotransmission. In vessels like the rabbit mesenteric artery, where noradrenaline and ATP cause synergistic constriction via α-adrenoceptors and P_{2x}-purinoceptors, respectively, in the rabbit coronary vessels the predominant effect of ATP is vasodilatation via P_{2y}-purinoceptors and noradrenaline also causes vasodilatation, by activation of β-adrenoceptors.

The question of how are noradrenaline and ATP stored is not yet totally solved. It is known that in spite of being costored they are not always released at a constant ratio (Stjärne, 1989). Prejunctional α_2-adrenoceptors and prejunctional P_1-purinoceptors seem to modulate the release of noradrenaline and ATP differentially: while activation of α_2-adrenoceptors reduces the release of noradrenaline to a greater extent than that of ATP (Driessen et al., 1993) the activation of P_1-purinoceptors reduces the release of ATP to a greater extent than that of noradrenaline (Driessen et al., 1994). Furthermore, isoprenaline, which causes β-adrenoceptor-mediated facilitation of the release of noradrenaline (Guimarães et al., 1978; Gonçalves et al., 1996) causes a β-adrenoceptor-mediated inhibition of neural release of ATP (Gonçalves et al., 1996). This differential modulation requires at least the existence of storage vesicles containing noradrenaline and ATP at different ratios.

Neuropeptide Y, another cotransmitter with noradrenaline and ATP, is also stored and released by sympathetic nerves (Lundberg et al., 1983, 1984). The contributions of noradrenaline, ATP and neuropeptide Y to neuronally evoked vasoconstriction differ depending on the frequency and duration of stimulation. Biochemical studies have demonstrated, in homogenates of sympathetic nerve endings, that noradrenaline, ATP and neuropeptide Y are stored in a heterogeneous population of storage vesicles: noradrenaline and ATP are present in both "light" and "heavy" particulate fractions while neuropeptide Y is present mainly in the "heavy" particulate fraction (Brock et al., 1998). It is likely that the "light" and "heavy" particulate fractions correspond respectively to the small and large dense cored vesicles identified morphologically in sympathetic nerve varicosities. To explain the findings with neuropeptide Y, it has been hypothesized that the contents of the small dense cored vesicles are preferentially released by low frequencies of stimulation, while at higher frequencies an increasing proportion of large dense cored vesicles release their contents (Brock et al., 1998).

Although it has direct vasoconstrictor actions in some vessels, the main action of neuropeptide Y is exerted at the prejunctional level and causes a negative modulation of noradrenaline release (Wahlestedt et al., 1986). It has been demonstrated that neuropeptide Y, via Y_1 receptors, plays a role in the long lasting vasoconstriction seen in some vascular beds after high frequency stimulation of sympathetic nerves in reserpine-pretreated pigs. This effect, which is inhibited by the selective Y_1 antagonists like BIBP3226 and SR120107A (Lundberg & Malmström, 1998), is much less evident in the presence of noradrenaline. However, even in untreated pigs, Y_1 receptor antagonists cause some reduction in the sympathetic vasoconstriction response in the renal circulation. In the pig spleen, circulating neuropeptide Y causes vasoconstriction via Y_2 receptors, whereas the neuronally released neuropeptide Y predominantly activates Y_1 receptors.

In most vessels, the non-α-adrenoceptor-mediated component of constriction caused by stimulation of sympathetic nerves is blocked by P_2-purinoceptor antagonists and accordingly has been taken to result from the release of ATP from the sympathetic nerve endings. Additionally, in some arteries and arterioles trains of prolonged high frequency stimulation evoke a contraction that is resistant to α-adrenoceptor and purinoceptor blockade. As this component is antagonized by Y_1-antagonists it appears to be due to released neuropeptide Y (Newhouse & Hill, 1997).

Role of Endothelium on Noradrenaline Release from Sympathetic Nerve Endings in Vascular Tissues

After Furchgott and Zawadzki (1980) had reported that the presence of the endothelium was required for acetylcholine to evoke relaxation in isolated rings of aorta, many biologically active substances were described which are produced by the endothelium and play important roles in the regulation of vascular smooth muscle tone

(Brutsaert & Andries, 1992; Hendersen et al., 1992). In experiments carried out on strips of rabbit pulmonary artery and on the isolated perfused heart of the rabbit, bradykinin was shown to reduce the overflow of noradrenaline elicited by electrical stimulation (Starke et al., 1977). More recently, indirect evidence was obtained supporting the view that the positive inotropic effect of bradykinin in rat atria and ventricle might be due to prejunctional facilitation of noradrenaline release (Minshall et al.,1994). In rat cardiac ventricle, it was shown that bradykinin concentration-dependently increases noradrenaline release evoked by electrical stimulation and this facilitatory influence disappeared after removal of the endocardium (Vaz-da-Silva et al., 1996). Similar results were obtained in the canine pulmonary artery: bradykinin markedly enhances noradrenaline overflow evoked by electrical stimulation (Guimarães et al., unpublished results). On the other hand, angiotensin II was shown to enhance noradrenaline release evoked by electrical stimulation (Guimarães et al.,1998[b]) but this effect was not affected by removal of the endocardium. Since bradykinin and angiotensin II have similar facilitatory effects on noradrenaline release evoked by electrical stimulation and are antagonized by the same antagonists at the same concentrations, the hypothesis was put forward that bradykinin acts on adrenergic varicosities through angiotensin II. All the results until now obtained confirm this hypothesis (Guimarães et al.,1998[c]).

All the other known endothelial products have either no effect or are negative modulators of noradrenaline release. While prostacyclin was shown to cause no change on adrenergic neuroeffector transmission (Vaz-da-Silva et al.,1996), both endothelin and NO cause a reduction of noradrenaline overflow elicited by electrical stimulation. The negative feedback modulation of endothelin was demonstrated at least in the guinea-pig pulmonary artery (Wiklund et al., (1989), in the canine mesenteric artery (Zhang et al.,1996) and in the rat tail artery (Mutafova-Yambolieva &Westfall, 1998), while that of NO was observed in the rat isolated perfused vasculature (Yamamoto et al., 1993; 1997).

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