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COMMENTARY

WHY ARE THERE SO MANY ADRENOCEPTOR SUBTYPES?

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Receptors are named, usually, after the endogenous substance that activates them, which implies that they participate in the physiological role of that substance. Adrenoceptors are considered to be the sites through which the two main natural catecholamines in mammals, adrenaline (their namesake) and noradrenaline, act as agonists. The neurotransmitter noradrenaline and circulating adrenaline and noradrenaline have a variety of physiological actions, particularly on the cardiovascular system, smooth muscle, and brown and white fat. Initial pharmacological studies differentiated these into α- and β-adrenergic responses and subsequently divided an α-adrenergic response into α_1 and α_2 subtypes. While these classifications remain valid today, both more detailed pharmacological characterization and biochemical and molecular biological analyses have indicated that each of the three broad classifications of adrenoceptors contains multiple individual gene products. Further subdivision has been suggested based on both basic pharmacological criteria and analysis of the pharmacological profile of molecularly defined species following expression in a range of cell types. However, such further potential subdivision may, in some instances, represent species variation in receptor subtype or differences in apparent ligand affinity due to expression in heterologous systems.

Pharmacological profile of adrenergic response subtypes

On the basis of pharmacological properties and rank order of potency for adrenaline (AD), noradrenaline (NA) and the synthetic agonist isoprenaline (ISO), adrenoceptors were divided into α and β. α-Adrenoceptors were defined as NA > AD $\gg$ ISO and β-adrenoceptors as ISO > AD = NA. α-Adrenoceptors were subdivided into α_1 and α_2, originally based on anatomical distribution, post-junctional α_1-adrenoceptors and pre-junctional α_2-adrenoceptors, although later an α_2 profile was seen for some post-junctional receptors [1]. The α_1-adrenoceptors are defined as stimulated by phenylephrine and blocked by prazosin (pA_2 8–11), and the α_2-adrenoceptors as stimulated by clonidine or UK-14,304 and antagonized by idazoxan (pA_2 7.2–8.5) or yohimbine (pA_2 7–9).

The wide range of potencies for prazosin seen in different tissues of the same species led to suggestions of heterogeneity within the α_1-adrenoceptor population [2]. Recent evidence suggests that α_1-adrenoceptors can be divided into at least four prazosin-sensitive ($pA_2 > 9.5$) subtypes (α_1A, α_1B, α_1C, α_1D; Table 1) and at least one subtype where prazosin shows lower affinity ($pA_2 < 9$; Table 1) [3–7].

Similarly, α_2-adrenoceptors have been further subdivided (α_2A, α_2B, α_2C, α_2D; Table 1) based on differences in ^{3}H-labeled antagonist binding and in the amino acid sequence and chromosomal location [8, 9]. Although three of these occur within the same species, it seems likely that the α_2A- and α_2D-adrenoceptors represent species homologues [9–12]. The main difference in the pharmacology, the lower affinity of yohimbine for the α_2D-adrenoceptor, may be due to a cys201ser change in the fifth transmembrane domain, as shown in Mα_2-10H human chimeras [13].

The possibility of heterogenous pre- versus post-junctional α_2-adrenoceptor subtypes is attractive, and the identification of the >100-fold post/pre-selective compound SK&F 104078 [14] appeared to support this heterogeneity, although this has since been disputed [15]. There is insufficient evidence to suggest which subtype shows higher affinity for SK&F 104078, α_2A, α_2B or α_2C, but the lower affinity for the α_2D has prompted the suggestion that this subtype may be pre-junctional [16].

β-Adrenoceptors (Table 1) have been subdivided into β_1- and β_2-adrenoceptors on the basis of the rank order of potencies of catecholamines acting on tissues producing different responses [17]: β_1 (ISO > NA > AD), and β_2 (ISO > AD > NA). This was confirmed by the development of the selective agonists dobutamine (β_1), salbutamol (β_2) and the antagonists CGP20712A (β_1) and ICI 118551 (β_2). A third subtype, the β_3-adrenoceptor, shows lower affinity for the agonists with a rank order NA > ISO > AD and low affinity for known β antagonists. As yet, no selective antagonists have been reported.

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Table 1. Characterization of multiple adrenoceptor subtypes

Mammalian subtype	Introns (human)	Chromosomal location (human)	Potency		Tissue distribution (rat unless indicated)	Major G protein	Effector system
			Agonist	Antagonist			
α_1A			OXY ≫ AD ≥ NA	Prazosin ($pA_2 > 9.5$) = WB-4101	Vas deferens, aorta [27]		↑ Phospholipase C [54,56]
α_1B [4]	1	5q32-q34	OXY > AD ≥ NA	Prazosin ($pA_2 > 9.5$) > WB-4101	Liver, brain	G_q [50]	↑ Ca^{2+} channel [3]
α_1C [5]	1	8	OXY ≫ AD ≥ NA	Prazosin ($pA_2 > 9.5$) = WB-4101	Olfactory bulb		↑ Phospholipase A_2 [54]
α_1D [6]		20p13	NA ≥ AD > OXY	Prazosin ($pA_2 > 9.5$) ≥ WB-4101	Vas deferens, brain		↑ Phospholipase D [55]
α_1L			NA > AD	WB-4101 = Prazosin ($pA_2 < 8.5$)	Aorta (guinea pig) [7]	?	?
α_2A [23]	0 [23]	10q24-q26	OXY ≫ AD ≥ NA	Yohimbine ≫ Prazosin	Platelet (human)		↓ Adenylyl cyclase [42, 44, 46, 47]
α_2B [25]	0	2	OXY = AD ≥ NA	Yohimbine > Prazosin	Liver, kidney	G_i [48]	↑ Phospholipase C, A_2 [47]
α_2C [24]		4	OXY = AD ≥ NA	Yohimbine > Prazosin	Brain, human kidney		↑ Phospholipase D [46]
α_2D			OXY ≫ NA	Yohimbine ≫ Prazosin	Brain, pineal gland (bovine) [16]		MAP kinase [48]
β_1 [18]	0 [28]	10q24-q26	ISO > NA ≥ AD	Betaxolol >> ICI 118551	Heart, pineal		
β_2 [19]	0 [19]	5q31-q33	ISO > AD > NA	ICI 118551 > Betaxolol	Lung, prostate	G_s [37, 38]	↑ Adenylyl cyclase [37]
β_3 [20, 35, 36]	1 [30]		NA > ISO > AD	Known β-antagonist weak	Adipose tissue/GI tract		↑ Ca^{2+} channel [38]
β-Atypical			NA > ISO > AD	Known β-antagonists weak	Adipose tissue/GI tract [22]		

The characteristics of the best-defined adrenoceptors in pharmacological, biochemical and molecular studies are shown in this table. The agonist potencies have been restricted to the endogenous agonists isoprenaline (ISO) and oxymetazoline (OXY) for comparison. Other abbreviations: NA, noradrenaline; and AD, adrenaline. Subtype-selective agonists are available for some but not yet for all the subtypes. Similarly, selective antagonists are available for α_1-, α_2-, β_1- and β_2-adrenoceptors but not for all the other subtypes. The data are compiled from Refs. 3–7, 16, 18–20, 22–25, 27, 28, 30, 35–38, 42, 44, 46–48, 50, 54–56 and from Lomasney JW and Allen L, Receptor nomenclature supplement, *Trends Pharmacol Sci*, 1993, as noted in the table.

Relationship of pharmacological profiles to molecular species

With the isolation of cDNA species corresponding to adrenoceptor subtypes, it has been possible to analyse how the pharmacological profiles of expressed molecularly defined receptors correspond with predictions based on classical pharmacology and how tissue and developmental profiles of expression are defined and regulated. Three distinct mammalian β-adrenoceptor cDNAs, β_1 [18], β_2 [19] and β_3 [20], have been isolated and, indeed, two distinct splice variant isoforms of the β_3-adrenoceptor, which vary by the presence or absence of six C-terminal amino acids, have been isolated (see below). Whereas expressed β_1- and β_2-adrenoceptor cDNA species display high similarities with pharmacological prediction, there are differences in the activities of certain β-adrenoceptor ligands on β_3-adrenoceptors expressed from cDNA in CHO cells and those expressed endogenously in adipose tissue [21]. While such studies indicate the possibility of β_3-adrenoceptor subtypes, it is not established and seems inherently unlikely, given the location of the splice variation, if this is explained simply by the existence of the two known splice variants. There is thus evidence that the "atypical" β-adrenoceptor is yet to be molecularly defined [22].

The existence of multiple isoforms of α_2-adrenoceptors produced from different genes on different chromosomes (α_2C10, α_2C4, α_2C2) is well established, but while the coincidence of the product of α_2C10 gene with the pharmacologically defined α_2A-adrenoceptor [23] has been firmly established for some time, there has been greater debate about the correlation of the α_2C4 [24] and α_2C2 [25] forms with the α_2B and α_2C adrenoceptors. It now appears that the α_2B adrenoceptor corresponds to the α_2C2 gene product and the α_2C adrenoceptor to the α_2C4 gene product. There has been further confusion centred on whether a fourth cDNA clone (rg20) corresponds to a separate (α_2D) isoform, but it seems that this product may be the rat homologue of the α_2A-adrenoceptor (see above).

There appear to be four distinct α_1-adrenoceptors, α_1A–D [4–6, 26]. Clones corresponding to α_1B–D are known, and while the cloned α_1D-adrenoceptor was initially thought to correspond to the pharmacologically defined α_1A-adrenoceptor, this, however, only refers to the atypical α_1A in the rat aorta [27].

Molecular analysis: genomic cloning

Genomic clones of both the β_2- and β_1-adrenoceptors have been isolated; these predict that both arise from intronless genes [28, 29], thus establishing that neither can generate diversity within these receptors by differential splicing. By contrast, genomic clones of the β_3-adrenoceptor from both humans [30] and rats [31, 32] indicate that their gene structure is more complex. In the case of the human β_3-adrenoceptor [30, 33], the gene consists of two exons and a single intron, whereas the rat gene consists of three exons and two introns. This clearly allows for the possibility of expression of multiple forms of the β_3-adrenoceptor. Indeed, polymerase chain reaction analysis has indicated that two different forms of this receptor are co-expressed in human adipose and intestinal tissue and that these differ by the presence or absence of a C-terminal six amino acid tail. Two distinct cDNA clones corresponding to these predictions have also been isolated [34–36]. The functional significance of these isoforms remains to be explored in detail. Both the α_2C10- and α_2C2-adrenoceptors are derived from intronless genes, whereas both the α_1B- and α_1C-adrenoceptor genes are more complex and contain at least one intron (although it has yet to be established whether this will lead to the generation of splice variant forms).

Signalling mechanisms

Although the four genetically distinct β-adrenoceptors may be coexpressed in individual cells and tissues, there is little reason to believe that they regulate different signalling systems. Each of the β-adrenoceptor subtypes is able to activate the stimulatory G-protein (G_s) and, hence, causes a stimulation of cyclic AMP accumulation. Despite this, there is clear evidence that the efficacy of the β_1-adrenoceptor to stimulate adenylyl cyclase is lower than that of the β_2 [37] and that this is an intrinsic property of the receptor rather than relating to the cell type in which the receptors are expressed. This presents an apparent paradox in that the β_1-adrenoceptor has been indicated to be the functionally predominant form in the heart, although in differing species the β_2-adrenoceptor may represent between 20 and 60% of the β-adrenoceptor population (see later). As activated G_s has also been reported to result in the regulation of calcium channels in the heart [38] (whether directly or via a cyclic AMP (cAMP)-dependent phosphorylation of the channel or both [39]), clearly β-adrenoceptors can thus also regulate cellular Ca^{2+} levels in a number of tissues. A further signalling function has been recorded for the β-adrenoceptor of turkey erythrocytes (which is pharmacologically most highly related to the mammalian β_1-adrenoceptor). As well as agonist stimulation of adenylyl cyclase, there is an activation of a phosphoinositidase C [40, 41]. The agonist profiles and effect curves for these two responses are equivalent, but it remains to be demonstrated unequivocally that both of these responses are produced via a single receptor subtype.

Signalling mechanisms associated with members of the α_2-adrenoceptor family are more complex. The classically accepted mechanism is to produce inhibition of adenylyl cyclase via stimulation of "G_i". In the cases in which it has been examined in detail, this has been shown to be via the G_i2 subtype [42, 43]. When the α_2C10 adrenoceptor was expressed at high levels in CHO cells, however, a potential ability to regulate adenylyl cyclase in a biphasic fashion was observed, as the receptor was shown to co-immunoprecipitate with both G_i and G_s and stimulation of adenylyl cyclase was shown to be produced by the receptor at high agonist concentrations while inhibition was produced with low levels of agonist [44]. Such observations are consistent with a hypothesis that the α_2C10-adrenoceptor couples selectively to G_i but that it also

exhibits a weaker interaction with G_s. Both the α_2C4- and α_2C2-adrenoceptors were also noted to interact with both G_i and G_s, but the interactive capacity for G_s, as assessed by the ability of the receptors to cause stimulation of adenylyl cyclase, showed selectivity in the order α_2C10 > α_2C4 > α_2C2 [44]. As such, the balance of regulation of adenylyl cyclase by α_2-adrenoceptors may reflect the cellular expression profile, the level of expression, and the concentration range of catecholamine to which the cell is exposed. Moreover, agonist activation of a variety of phospholipases (phosphoinositidase C [45], a phosphatidylcholine-directed phospholipase D [46] and phospholipase A_2 [47]) and regulation of $p21^{ras}$ and the mitogen-activated protein (MAP) kinase cascade [48] have also been recorded for α_2-adrenoceptors. The reports of such effects tend to have been produced from studies in which a relatively high-level expression of an α_2-adrenoceptor has been achieved by either transient transfection or stable transfection into fibroblast cell lines. In the majority of these cases, the regulation of the phospholipase effector cascade is blocked by pretreatment of the cells with pertussis toxin, suggesting by analogy with other systems that the effect may be produced by the $\beta\gamma$ subunits associated with $G_i\alpha$ [49]. There is insufficient information to assess (a) if the individual α_2-adrenoceptors differ significantly in their ability to activate these cascades, or (b) the potential physiological relevance of these actions.

Agonist activation of the α_1-adrenoceptors is usually anticipated to regulate the intracellular levels of inositol 1,4,5-trisphosphate and diacylglycerol via activation of a phosphoinositidase C. Again by both transient and stable expression of different molecular forms of these receptors, it has been noted that the α_1C-adrenoceptor couples more efficiently to this pathway than does the α_1B-adrenoceptor [5]. In transfected cell systems, this has been shown to involve the participation of the pertussis toxin-insensitive G-proteins, G_q and/or G_{11} [50]. However, a 74 kDa pertussis toxin-insensitive G-protein designated G_h has also been suggested to play a role in pertussis toxin-insensitive effects of α_1-adrenoceptors [51], and a variety of α_1-adrenoceptor effects have been reported to be attenuated by pertussis toxin treatment of cells and tissues [52]. These observations imply the interaction of α_1-adrenoceptors with a range of G-proteins [53], but there is no evidence at this stage to indicate that this will correlate with different α_1-adrenoceptor subtypes or their function.

Further second messenger effects of α_1-adrenoceptors, which have been recorded, include stimulation of phospholipase A_2 activity [54], hydrolysis of phosphatidylcholine [55] and inhibition of cellular cAMP levels, although this effect is likely to represent the activation of a cAMP phosphodiesterase rather than direct inhibition of adenylyl cyclase [56].

Desensitization and paradoxical regulation

Paradoxical regulation. Analysis of the 5′ flanking region of the β_3-adrenoceptor indicates that it contains a number of potential cAMP response elements, suggesting that the expression of this receptor might be positively regulated by cellular levels of cAMP.

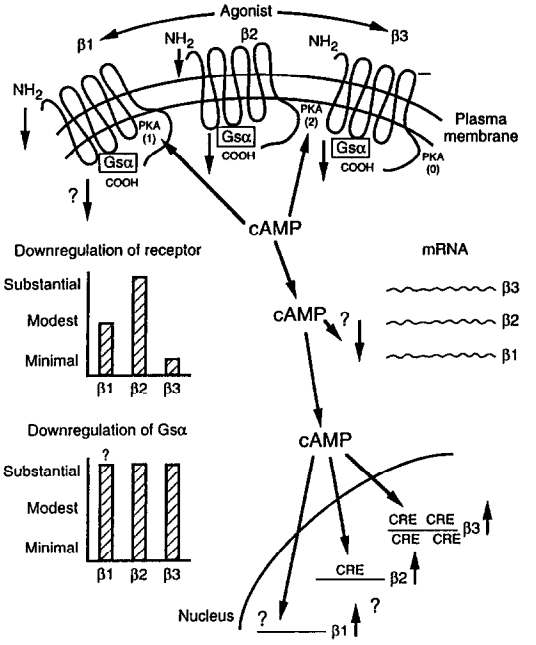

Fig. 1. Agonist-induced regulation of β-adrenoceptor subtype density and function: The role of cAMP. Agonist activation of β_1-, β_2- or β_3-adrenoceptors results in reduced levels (↓) of both β_1 and β_2 but not β_3 (−). If there are high levels of either the β_2- or β_3-adrenoceptor, this can result in a substantial reduction of cellular levels of $G_s\alpha$ (↓). It is likely, but has not been formally demonstrated, that the same is true for the β_1-adrenoceptor. cAMP generated by agonist activation of any of the β-adrenoceptor subtypes can result in up-regulation (↑) of expression of at least both the β_3- and β_2-adrenoceptor genes. A cAMP-dependent mechanism can also down-regulate (↓) levels of, at least, β_2-adrenoceptor mRNA, leading to reduced synthesis of the polypeptide. There are, respectively, one, two and no consensus sites in the β_1-, β_2- and β_3-adrenoceptor primary sequences for cAMP-dependent protein kinase. These phosphorylations act to "uncouple" the receptor from G_s and hence can generate varying degrees of both homologous and heterologous desensitization within this receptor family. The question mark (?) indicates elements that are either likely but have not been demonstrated directly or for which the evidence is not unambiguous. Abbreviations: PKA, protein kinase A; and CRE, cAMP response element.

In 3T3-F442A cells differentiated towards an adipocyte phenotype by the addition of insulin, the β_3-adrenoceptor is expressed although it is not in the undifferentiated fibroblast phenotype [57]. These cells also express the β_1-adrenoceptor at low levels in the fibroblastic form, and levels of mRNA encoding this polypeptide are also increased with differentiation. When differentiated cells were exposed to isoprenaline, levels of β_3-adrenoceptor mRNA were found to be elevated within 4 hr and this was maintained for at least 30 hr; in addition, levels of the β_3-adrenoceptor also increased with a similar temporal pattern. However, in parallel, the levels of the β_1-adrenoceptor declined by some 70% [58]. mRNA encoding the β_2-adrenoceptor has also

been detected in differentiated 3T3-F442A cells [57]. A cAMP-responsive element has also been noted in the promoter region of the β_2-adrenoceptor, and dexamethasone (presumably via activation of a glucocorticoid response element) causes a significant increase in the levels of β_2-adrenoceptor message in these cells, particularly if the glucocorticoid was supplied to the undifferentiated cells and its presence maintained during the differentiation process [57]. By contrast, dexamethasone treatment of preadipocyte 3T3-F442A cells causes a complete depletion of β_1- and β_3-adrenoceptor mRNAs. It is thus clear that complex, gene-specific regulation of members of the β-adrenoceptor family can occur in a single cell in response to individual stimuli [21]. Such information demonstrates that individual cell types may be able to regulate their β-adrenoceptor profile, and as single ventricular myocytes have been shown to co-express β_1- and β_2-adrenoceptors [59], this may provide an important control mechanism (Fig. 1). However, while intriguing in its own right and suggestive of the potential for specific temporal and developmental regulation of these receptors, this does not in isolation define why multiple genetic forms of the receptor are required if each is used to regulate the same signalling pathway (see later).

An apparent paradoxical cross-regulation of adrenergic responsiveness between β_1- and β_2-adrenoceptors is indeed noted in cardiac tissue of patients treated with β_1-selective antagonists. Such treatment results in a sensitization of response to catecholamines at the β_2-adrenoceptor but not at the β_1-adrenoceptor [60, 61]. The mechanism(s) responsible for this phenomenon is unidentified but does not include a selective up-regulation of the amount of the β_2-adrenoceptor. The functional relevance of coexpression of β_1- and β_2-adrenoceptors and such cross-regulation of receptor sensitivity in the heart are considered later.

Desensitization. A common pattern of regulation for many G-protein-linked receptors in response to short-term exposure to an agonist involves phosphorylation of the receptor at a number of sites. This has been examined most completely for the β_2-adrenoceptor [62, 63]. This receptor has a number of potential phosphorylation sites, primarily within the C-terminal tail of the receptor but also within the third intracellular loop. In the case of the β_2-adrenoceptor, both protein kinase A and a receptor kinase that is highly selective for the agonist-occupied form of the receptor, termed β-adrenoceptor kinase (BARK), play definite roles. As in many systems β-adrenoceptor agonist-mediated stimulation of adenylyl cyclase is noted to be produced with a concentration dependence that is to the left of the receptor occupancy by the agonist, then activation of protein kinase A and hence phosphorylation of sites within the β_2-adrenoceptor by this kinase can occur at lower levels of agonist than that by BARK. Activation of BARK follows receptor occupancy curves closely, since this kinase acts only on the agonist-occupied form of the receptor [63–66]. The result of such modifications is a rapid "uncoupling" of the receptor from $G_s\alpha$ upon exposure of a cell expressing this receptor to an agonist. Whether this process involves a physical separation of the receptor and G-protein is not entirely clear, but as there appears to be a central role of G-protein $\beta\gamma$ subunit in attracting BARK, which in the resting state is a primarily cytoplasmic enzyme, to the plasma membrane [67, 68] where it can now act upon the β_2-adrenoceptor, it must be assumed that at least the $\beta\gamma$ subunits of G_s remain in close proximity to the receptor. Serine 262 (a target for phosphorylation by protein kinase A) also plays a key role in rapid "uncoupling" of the receptor from G_s [69].

Longer term exposure to an agonist involves, initially, a sequestration of the receptor to a location that may represent a vesicular pool, the nature of which remains poorly defined. In this state, the receptor is accessible in whole cell binding studies to hydrophobic but not to hydrophilic receptor ligands. Subsequently, if the presence of the agonist is maintained, down-regulation occurs. The phenomenon of down-regulation is a reflection of a variety of processes that include both an enhancement of protein degradation and a destabilization of mRNA encoding the receptor [70], both of which, in the absence of other regulatory processes, would be anticipated to result in a time-dependent reduction in total cellular levels of the receptor. Evidence from mutational analysis indicates that both of two tyrosine residues in the C-terminal tail of the receptor (Tyr 350 and Tyr 354) are important for agonist-mediated down-regulation [71, 72], but the details of their role remain to be fully addressed. Whereas both the β_1 (one site)- and β_2 (two sites)-adrenoceptors have sequence motifs consistent with their acting as substrates for phosphorylation by protein kinase A, the same is not true of the β_3-adrenoceptor (Fig. 1). Indeed, in this regard it is interesting to note that the β_3-adrenoceptor is resistant both to agonist-mediated short-term desensitization [73] and, on a longer time scale, to agonist-induced receptor down-regulation. Thus, in cells expressing a mixed complement of β-adrenoceptor subtypes, the quantitative importance of signalling from β_1- and β_2-adrenoceptors might be anticipated to decline with time, whereas that of the β_3-adrenoceptor would increase.

Since all the β-adrenoceptor subtypes activate G_s and thence adenylyl cyclase, then regulation in levels or the activity of these polypeptides might be anticipated to provide a common means of heterologous desensitization for all co-expressed β-adrenoceptor subtypes. Indeed, β_2-adrenoceptor-mediated down-regulation of cellular $G_s\alpha$ levels has been shown to occur in an agonist and receptor-level dependent manner [74]. While there are known to be four potential splice variants of $G_s\alpha$ [75], and the steady-state ratios of expression of the pairs of long and short forms of this G-protein can vary considerably between cells, there is little current evidence to suggest that individual receptors interact differently with them [76]. While a potential phosphorylation site is determined by the exact splice acceptor site utilized [75], and forms of $G_s\alpha$ have been reported to act as substrates for both protein kinase A [77] and protein kinase C [78] *in vitro* evidence for the agonist-mediated regulation of phosphorylation of this species *in vivo* remains fragmentary. Similarly, while phosphorylation of adenylyl cyclase in a

protein-kinase C-dependent manner was reported in frog erythrocytes [79] some time ago, the concept that different isoforms of adenylyl cyclase may be regulated by different β-adrenoceptor subtypes has not yet been examined. However, the potential for cross-regulation of β-adrenoceptor function in heart by α_1-adrenoceptor-mediated activation of protein kinase C, and hence inactivation of adenylyl cyclase, is intriguing given the interaction of α_1- and β-adrenoceptors in the control of force of contraction in the heart (see below). Furthermore, sustained exposure of rat heart muscle cells to noradrenaline has been reported to elevate cellular levels of G_i [80], and this correlates with a heterologous desensitization of receptors that function to stimulate adenylyl cyclase. As such, in heart failure, in which prolonged exposure to increased levels of noradrenaline may occur, increasing levels of G_i [81] may restrict the activity of β-adrenoceptor subtypes.

There is also evidence that different adrenoceptors undergo different patterns or pathways of intracellular sorting following exposure to agonists. Following short-term exposure to agonist of cells transfected to express either β_2-, α_2C10- or α_2C4-adrenoceptors, the β_2-adrenoceptors were internalized to a population of intracellular vesicles distinct from those containing the α_2C4-adrenoceptor. Over this time scale, the α_2C10-adrenoceptor remained at the plasma membrane [82].

As with the β_1- and β_2-adrenoceptor subtypes, evidence indicates that agonist-induced phosphorylation of sites within the third intracellular loop of some α_2-adrenoceptor subtypes is likely to play an important role [83]. Expression of each of the α_2C10-, α_2C4- and α_2C2-adrenoceptors in CHO cells and short-term challenge with agonist resulted in functional desensitization of both the α_2C10- and α_2C2-adrenoceptors but no effect on the α_2C4-adrenoceptor. Moreover, the α_2C4-receptor was resistant to down-regulation during sustained exposure to agonist, whereas both the α_2C10- and α_2C2-adrenoceptors were down-regulated [84]. Even without down-regulation of the α_2C4-adrenoceptor, agonist-induced down-regulation of G_i was noted to occur [84] and may be responsible for the degree of long-term desensitization noted with sustained agonist exposure at this receptor [84, 85].

The general concept that G-proteins are down-regulated following sustained exposure of cells to an agonist for a receptor linked to that G-protein has gained credence recently [85]. In addition to the situation with the α_2-adrenoceptor subtypes mentioned above, this has been noted to occur for both the β_2- [74] and β_3-adrenoceptor (Milligan *et al.*, unpublished observations) and for the α_1B- and α_1C-adrenoceptors (Milligan *et al.*, unpublished observations) and clearly contributes to the patterns of sustained desensitization (Fig. 1).

Cardiovascular control

The sympathoadrenal (mainly adrenergic) system is a major regulator of cardiovascular control [for review, see Ref. 86]. Cardiac adrenoceptors are located on several different cell types and mediate diverse effects. No longer are β_1-adrenoceptors considered to be the exclusive adrenoceptor population through which catecholamines exert their effects on cardiac muscle. α_1-, α_2-, β_2- and β_3-Adrenoceptors (Fig. 2) have all been identified on myocytes [87–89], although the function and subtype(s) of the α_2-adrenoceptor(s) are as yet unknown. Intriguingly, in the heart β_1-, β_2- and to some extent β_3-, α_1A- and α_1B-adrenoceptors mediate effects that are functionally similar, suggesting the possibility of cross-regulation of adrenoceptor sensitivity. Under normal conditions, noradrenaline preferentially acts on the β_1-adrenoceptor (>75% of the response), thereby increasing cAMP and initiating a signal cascade resulting in cardiac contraction. However, in the compromised heart, the β_2-adrenoceptor, which represents a significant percentage of the β-adrenoceptor population (25–60% of the human heart; [90–92]) and is associated with increased inotropic effects *in vitro* [92] and increased chronotropic effects *in vivo* [93], may be functionally more sensitive to noradrenaline. Indeed, patients on long-term β_1-adrenoceptor antagonists show unaltered β_1 responses (after wash-out of the β_1 blocker), whereas β_2 responses are potentiated (>6 fold) *in vitro* and *in vivo* [60, 61]. Although the cardiac β_3-adrenoceptor [89] has also been shown to have inotropic or lusitropic function in healthy volunteers [94], its role in the diseased heart remains to be evaluated.

Acute stimulation of α_1-adrenoceptors modulates various steps of the cardiac excitation–contraction coupling cascade and has been shown to be involved in inotropic and chronotropic responses and cardiac conduction [53]. The relative numbers of the α_1-adrenoceptor subtypes in the myocardium have been determined for the α_1A and α_1B subtypes: rat α_1A:α_1B, 20:80% [95]; rabbit α_1A:α_1B, 37:63% [88]; similarly in the dog there is a higher proportion of chloroethylclonidine (CEC)-sensitive α_1B sites [96]. Other non-α_1A or -α_1B subtypes have been identified in the heart [97], but their relation to known subtypes has not been defined.

Stimulation of myocardial α_1-adrenoceptors produces a positive inotropic response. However, demonstration of the small α_1-adrenoceptor-mediated component in the normal heart requires either β_1-antagonism or the use of a highly selective α_1-agonist. Chronic phenylephrine-induced inotropy (in the presence of β-block) is abolished by pre-treatment with CEC, suggesting that this is an α_1B subtype functional response [88], although, at least in the rat, the α_1-adrenoceptor-mediated positive inotropic effect in the papillary muscle is via the α_1A subtype [98].

α_1-Adrenoceptor agonists have also been shown to increase the duration of the action potential [99, 100], and to be more effective on atrial than ventricular muscle [101]. The subtype involved is not known, but it could be the α_1A subtype as it shows sensitivity to WB-4101 [102]. The α_1A subtype acts to restore Ca^{2+}-dependent (slow) action potentials, i.e. increases the Ca^{2+} inward current.

Multiple adrenoceptors in the heart allow for endogenous control via changes in receptor density and sensitivity. The α-adrenoceptor response is more important in disease states where the balance of α–β-adrenoceptors in the heart is altered in favour

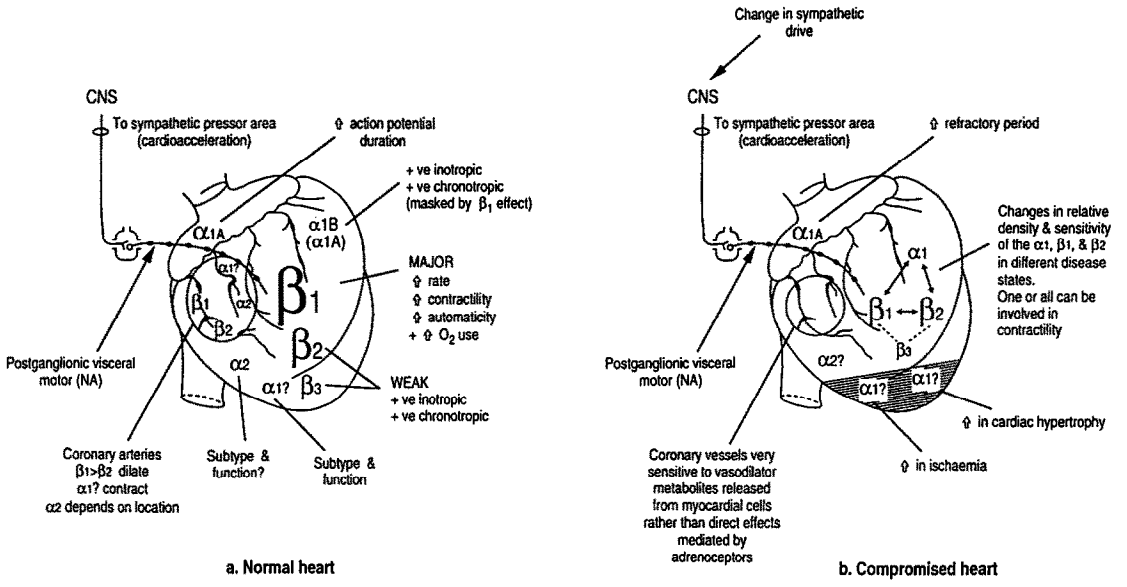

Fig. 2. Multiple adrenoceptors in the heart. In the normal heart (a), the majority of the noradrenaline response (>75%) activates β_1-adrenoceptors (indicated by the large β_1 symbol), resulting in positive chronotropic and inotropic effects, increased automaticity and facilitation of conduction in the AV node with a corresponding decrease in cardiac efficency. However, β_2, β_3 and α_1 (α_1A and/or α_1B) can also produce positive inotropic and/or chronotropic responses. As the number and sensitivity of the different subtypes are known to change in different pathological conditions (b; also see text), this would suggest that different subtypes can be utilized. The β-adrenoceptors activate G_s, which results in opening of calcium channels either directly or via a cAMP-dependent phosphorylation of the channel or both. The contribution of each subtype to the overall response will depend on the receptor number, sensitivity to noradrenaline, and efficacy of the receptor subtype to stimulate adenylyl cyclase. The α_1-adrenoceptor-mediated positive inotropic response is unrelated to cAMP; it remains to be determined how the responses of the α- and β-adrenoceptors are cross-regulated at the second messenger level. It is likely that multiple subtypes in the heart act as a "catecholamine backup system," which can respond in the event of β_1-adrenoceptor failure. Other subtypes (α_2, α_1?) have been identified in the normal heart (a), but their function is unknown. Changes in the number of α_1-adrenoceptors may be important in other pathologies of the heart, including ischaemia and hypertrophy (b), but the subtypes involved and the mechanisms require further study.

of α and β_2, which may offer the heart a catecholamine-sensitive backup in the event of β_1-adrenoceptor failure. Changes occur following chronic treatment with β_1-antagonists ([103] increase in α_1 and [60, 61] increase in β_2 sensitivity), congestive heart failure ([104] decrease in β_1), cardiac hypoxia ([105] increase in α_1), hypertension in animals ([106] decrease in β_1) and in hypothyroidism ([107] increased inotropic response to α_1-adrenoceptor agonists) and diabetes ([108] increased inotropic response to α_1-adrenoceptor agonists). There is also an increase in α_1-adrenoceptor number in ischaemia, induced by increased acyl carnitine levels [109, 110] and following cardiac hypertrophy [111, 112]. α_1-Adrenoceptor blockers may protect the ischaemic myocardium by blocking these externalized receptors, and the calcium entry blockers may protect against the increased Ca^{2+} sensitivity induced by increased α_1-adrenoceptor activity [113, 114].

Blood flow

Catecholamines mediate both contraction and relaxation of vascular smooth muscle, which immediately implies the need for two subtypes; in the main, α-adrenoceptors contract smooth muscle via the release of intracellular calcium and β-adrenoceptors cause relaxation by initiating an increase in cAMP and subsequent upstream events. α_1-Adrenoceptors are present throughout the vasculature, although they are more prominent on the arterial side. Preliminary pharmacological studies suggest that a number of subtypes are present; the α_1A-adrenoceptor is the predominant receptor on renal arteries, whereas both α_1A- and α_1B-adrenoceptors are present on both mesenteric artery and portal vein. The important hypertensive α_1-adrenoceptor on rat aorta has been difficult to classify, but molecular studies show that the aorta expresses the α_1D [6] (atypical α_1A [27]) and α_1B subtypes. This suggests that the α_1A, α_1B and particularly the α_1D subtypes are involved in determining peripheral resistance. It is likely that α_2-adrenoceptors are present in all but the largest of arteries, although more abundant on venous smooth muscle and superficial resistance arteries. However, the effects of post-junctional α_2-adrenoceptors are often masked by pre-junctional α_2-adrenoceptors (possibly the α_2D subtype, see above)

mediating noradrenaline release and because *in vitro* the α_1-adrenoceptors dominate functionally. However, by changing the experimental conditions to more like that seen *in vivo*, tonically activating the system with a non-α-adrenoceptor stimulant, post-junctional α_2-adrenoceptors become functionally evident [115–118]. Therefore, the possibility remains that α_2-adrenoceptors play a role in venous capacitance and that α_2-adrenoceptor antagonists could be used as peripheral dilators of the extremities; indeed an increase in α_2-adrenoceptor function has been reported in Raynaud's disease [119].

More selective compounds are needed to identify which subtypes are present on different vessels and hence which α_2-adrenoceptor subtypes are important in the control of blood flow. The α_2A-adrenoceptor may be responsible for the resistance to salt-induced hypertension, as it is deficient in the kidneys of salt-sensitive Sabra rats [120].

In contrast, the β_1- and β_2-adrenoceptors dilate blood vessels, the β_2-adrenoceptor response being dominant except on coronary arteries where β_1-adrenoceptors relax smooth muscle. In addition to the β_1 response, activation of α_2-adrenoceptors on coronary vascular smooth muscle of dog and pig can release endothelium-derived relaxant factor (EDRF) with a consequent relaxation of the pre-contracted vessel [121]. Release of EDRF has also been reported in canine pulmonary and femoral arteries and veins but varies in relation to species and blood vessel. Thus, α_2-adrenoceptors can have opposing effects on vascular tone: the post-junctional subtype on vascular smooth muscle cells contract smooth muscle activated by neuronal noradrenaline, whereas the pre-junctional subtype acts to oppose this by a negative feedback on noradrenaline. In addition, those on the endothelium relax smooth muscle in response to circulating adrenaline due to release of EDRF. Thus far, no studies have described different subtypes for these effects and no selective compounds, except perhaps the post-selective compound SK&F 104078 [14] although, as discussed above, this is disputed [15].

Brown adipose tissue

In brown adipose tissue, the same single stimulus, noradrenaline, promotes many, if not all, physiological functions. The main and perhaps the only physiological function of the mature, fully differentiated brown adipocyte is thermogenesis. This is produced via the generation of cAMP [122], which stimulates lipolysis and respiration and at the level of inner mitochondrial membrane results in an increased proton conduction. The dissipation of the electrochemical gradient of protons results in generation of heat [123]. However, prior to the ability of brown adipose cells to act as a thermogenic tissue, recruitment processes including growth, development and functional specialization have to occur in the immature cells [124].

This temporal variation in the effects that must be produced by noradrenaline may explain why the same cell expresses different β-adrenoceptor subtypes [125] even though they act via the same signal transduction pathway. If we assume that a different β-adrenoceptor subtype is used to initiate cellular development prior to thermogenic function, then it would be appropriate if the receptor mediating development became desensitized or down-regulated, whereas the receptor subtype required for thermogenic responses should be maintained in an active state or up-regulated during development.

Brown fat expresses a number of distinct adrenoceptor subtypes [126–129]. However, except in some pathophysiological conditions, the contribution of β-adrenoceptors is primary.

If the maturation and physiological function of brown adipose tissue are viewed as a sequence in which the β_1-adrenoceptor is responsible for DNA synthesis and cellular proliferation and the β_3-adrenoceptor for cAMP-induced lipolysis, respiration and thermogenesis, then a desensitization of the β_1-adrenoceptor would be required to stop proliferation and quantitative growth, whereas the β_3-adrenoceptor would have to be newly synthesized or able, somehow, to avoid initial noradrenaline-mediated desensitization. We have described previously (see above) the paradoxical regulation of β_1- and β_3-adrenoceptors during differentiation of preadipocyte 3T3-F442A cells and the resistance of β_3-adrenoceptors to short-term desensitization and down-regulation. Such characteristics make this receptor subtype ideally suited for this function, both in terms of genetic control of its synthesis and in terms of regulation, as the β_3-adrenoceptors would be expected to remain active even when co-expressed with the β_1-adrenoceptor. The β_3-adrenoceptor also shows low affinity for noradrenaline and adrenaline in comparison to β_1- and β_2-adrenoceptors. Furthermore, the β_3-adrenoceptor may be present in large quantities in comparison to the β_1-adrenoceptor [130]. Under such conditions, noradrenaline at low concentrations (1–10 nM) [122] would activate the low-capacity, high-affinity β_1-adrenoceptors, whereas later, higher concentrations of noradrenaline would stimulate the low-affinity, high-capacity β_3-adrenoceptors.

As noted above, agonist-stimulated G-protein down-regulation occurs in a variety of cells and tissues [85]. However, recent data indicate that effective down-regulation of a G-protein will occur only if a significant portion of the overall pool of that G-protein is activated [74, 131]. Furthermore, $G_s\alpha$ down-regulation, in contrast to that of the β-adrenoceptors [70], is independent of the generation of cAMP [49]. Thus, agonist occupation of the low levels of β_1-adrenoceptors in brown fat is unlikely to cause down-regulation of a significant fraction of $G_s\alpha$. This is relevant as $G_s\alpha$ levels presumably must be maintained to allow generation of the subsequent β_3-adrenoceptor response for thermogenesis unless the receptors interact selectively with different $G_s\alpha$ isoforms.

Conclusions

It appears that although there are already many known adrenoceptor subtypes, there is the need for all and possibly more. Multiple subtypes allow for multiple functions in different tissues. Where receptor subtypes are associated with the same function, the contribution of each subtype to the overall response will depend on the receptor number,

sensitivity to noradrenaline or adrenaline, efficacy of the receptor subtype to couple to the second messenger system, and on the regulation, expression and degradation of the receptor polypeptide. This may, of course, vary in pathological conditions allowing for the possibility of a backup catecholamine-sensitive system, i.e. if the function of one receptor subtype is compromised, another can function, as seen with inotropic and chronotropic responses in the heart.

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