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Review

α_1-Adrenoceptor subtypes

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Abstract

α_1-Adrenoceptors are one of three subfamilies of receptors (α_1, α_2, β) mediating responses to adrenaline and noradrenaline. Three α_1-adrenoceptor subtypes are known (α_{1A}, α_{1B}, α_{1D}) which are all members of the G protein coupled receptor family, and splice variants have been reported in the C-terminus of the α_{1A}. They are expressed in many tissues, particularly smooth muscle where they mediate contraction. Certain subtype-selective agonists and antagonists are now available, and α_{1A}-adrenoceptor selective antagonists are used to treat benign prostatic hypertrophy. All subtypes activate phospholipase C through the $G_{q/11}$ family of G proteins, release stored Ca^{2+}, and activate protein kinase C, although with significant differences in coupling efficiency ($\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$). Other second messenger pathways are also activated by these receptors, including Ca^{2+} influx, arachidonic acid release, and phospholipase D. α_1-Adrenoceptors also activate mitogen-activated protein kinase pathways in many cells, and some of these responses are independent of Ca^{2+} and protein kinase C but involve small G proteins and tyrosine kinases. Direct interactions of α_1-adrenoceptors with proteins other than G proteins have not yet been reported, however there is a consensus binding motif for the immediate early gene Homer in the C-terminal tail of the α_{1D} subtype. Current research is focused on discovering new subtype-selective drugs, identifying non-traditional signaling pathways activated by these receptors, clarifying how multiple signals are integrated, and identifying proteins interacting directly with the receptors to influence their functions.

Keywords: Adrenoceptor; Noradrenaline; Adrenaline; Mitogenic signaling; Ca^{2+}

1. Introduction

Noradrenaline and adrenaline play important roles as neurotransmitters and hormones throughout the body. The adrenoceptors through which these compounds act are targets for many therapeutically important drugs. The adrenoceptors are subdivided into 3 families (α_1, α_2, β) based on their pharmacology, structure, and signaling mechanisms (Fig. 1; Bylund et al., 1994). Each family contains three or more subtypes, all of which are members of the G protein coupled receptor superfamily. These receptors consist of single polypeptide chains predicted to have 7 membrane spanning domains. The α_1-adrenoceptor family is of particular therapeutic interest because of its important role in control of blood pressure (Piascik et al., 1990; Bylund et al., 1994; Minneman and Esbenshade, 1994). These receptors are also abundant in brain, where their functional role is not yet clear, and play critical roles in controlling contraction and growth of smooth and cardiac muscle.

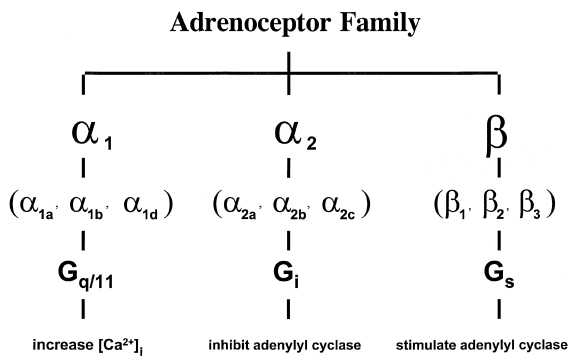

Fig. 1. The adrenoceptor family is divided into three subfamilies based on pharmacological properties, structural homology, and signaling mechanisms.

2. Existence of pharmacologically distinct α_1-adrenoceptor subtypes

The first strong evidence for pharmacologically distinct α_1-adrenoceptor subtypes came from studies of [^{3}H]-

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prazosin binding to rat brain membranes (Morrow and Creese, 1986). Inhibition of [^{3}H]-prazosin binding by WB 4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4 benzodioxane) and phentolamine, but not by a variety of other antagonists, was characterized by a relatively shallow slope, indicating binding site heterogeneity. Morrow and Creese (1986) concluded that two distinct α_1-adrenoceptor subtypes were labeled by [^{3}H]-prazosin, and they named the site with a higher affinity for WB 4101 and phentolamine α_{1A}, and the other site α_{1B}. It was subsequently recognized that the site-directed alkylating agent chloroethylclonidine inactivated only a subpopulation of α_1-adrenoceptor binding sites in membranes from a variety of tissues (Han et al., 1987a). Since chlorethylclonidine-insensitive sites correlated well with those with a high affinity for WB 4101 (Minneman et al., 1988), it was concluded that these two approaches were distinguishing the same α_{1A} and α_{1B} subtypes. α_{1A}-adrenoceptor selective antagonists with a greater degree of selectivity than WB 4101 were then identified, including 5-methylurapidil (Gross et al., 1988) and (+)-niguldipine (Boer et al., 1989). These drugs were shown to discriminate between the same subtypes, supporting the existence of discrete α_{1A} and α_{1B} subtypes (Han and Minneman, 1991).

3. Cloning, structure, and splice variants

It is now clear that there are at least three α_1-adrenoceptor subtypes. Although only two subtypes (α_{1A} and α_{1B}) were easily distinguished pharmacologically, there was some evidence for additional heterogeneity (Minneman, 1988). Following the original cloning of the hamster α_{1B}-adrenoceptor by Cotecchia et al. (1988), two additional cDNAs have been cloned (Schwinn et al., 1990; Lomasney et al., 1991; Perez et al., 1991). For complicated reasons, the relationship between the pharmacologically defined subtypes and the cDNA clones was controversial for several years. Following the cloning and analysis of the human receptors (Ramarao et al., 1992; Hirasawa et al., 1993; Forray et al., 1994; Esbenshade et al., 1995; Schwinn et al., 1995; Tseng-Crank et al., 1995), and identification of more selective drugs (Goetz et al., 1995), however, a general consensus has been reached (Ford et al., 1994; Hieble et al., 1995). The clone originally named α_{1C} has now been shown to encode the α_{1A} subtype (Laz et al., 1994; Perez et al., 1994), and the clone originally named $\alpha_{1A/D}$ has been shown to encode a novel subtype with unique pharmacological properties, the α_{1D} (Fig. 2; Esbenshade et al., 1995; Hieble et al., 1995). Thus, it now appears that there are three subtypes encoded by three genes (α_{1A}/α_{1a}, α_{1B}/α_{1b}, α_{1D}/α_{1d}), where upper and lower case letters indicate pharmacologically defined and cloned subtypes, respectively. Because of the initial confusion in nomenclature, the α_{1C} designation has been dropped. There is functional evidence for further heterogeneity, particularly for a subtype with a low affinity for prazosin (Muramatsu et al., 1990; Garcia-Sainz et al., 1992; Hieble et al., 1995; Ford et al., 1996; Leonardi et al., 1997). However, to date these putative additional subtypes have resisted identification by biochemical and/or molecular techniques.

The α_1-adrenoceptor subtypes are unusual among G protein coupled receptors in that they have an intron in their protein coding regions at the end of the putative 6th transmembrane domain (Ramarao et al., 1992; Tseng-Crank et al., 1995; Chang et al., 1998). Three splice variants of the human α_{1A}-adrenoceptor have been cloned which dif-

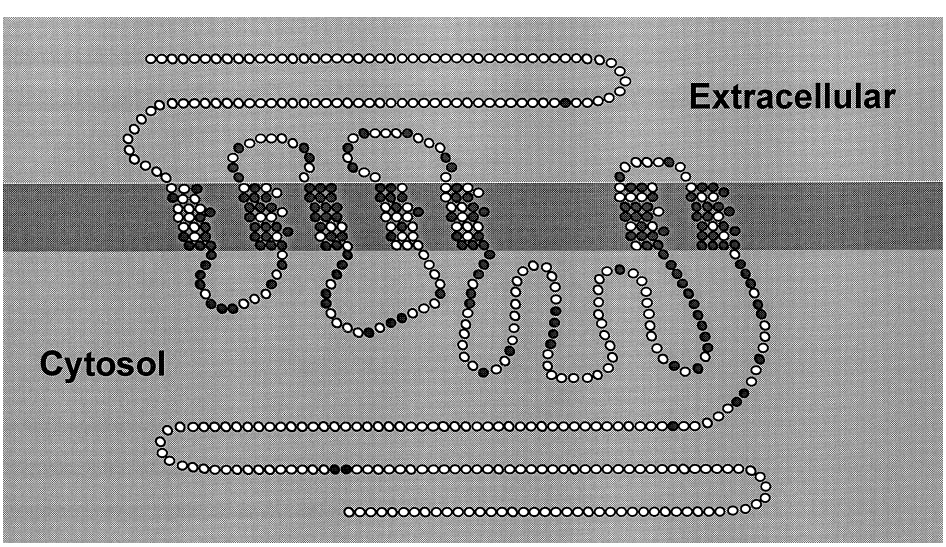

Fig. 2. A hypothetical representation of the structure of the α_{1D}-adrenoceptor. The receptor is a single polypeptide, predicted to have seven domains that span the plasma membrane. Amino acid residues that are identical to the other two α_1-adrenoceptor subtypes are shown as filled circles.

fer in length and sequence of their C-terminal domains (Hirasawa et al., 1995), and additional splice variants and truncated products have been reported (Chang et al., 1998). However, no pharmaco-logical or signaling differences are observed on expression of these different splice variants. The truncated products do not bind radioligand, and their biological significance is still obscure (Chang et al., 1998).

4. Expression and distribution

Radioligand binding assays have shown that α_1-adrenoceptors are expressed in a large number of tissues from a number of species. Unfortunately, specific antibodies to the three α_1-adrenoceptor subtypes are still not available, and the lack of highly subtype-selective antagonists makes quantitative analysis by radioligand binding methods difficult. Thus, mapping the distribution of these subtypes has been performed largely by analysis of mRNA expression patterns. Mapping studies have been performed primarily in humans and rats, although some information from other species has also been published.

High expression levels of all three subtypes are found in brains of both species, although there is substantial variation in subtype expression between brain regions (Price et al., 1994a,b; Rokosh et al., 1994; Scofield et al., 1995). All three subtypes are also expressed in heart, although the α_{1A} may be the dominant cardiac subtype in humans (Price et al., 1994b). Vas deferens and other smooth muscles appear to express predominantly α_{1A} and α_{1D}, although some α_{1B}-AR message and protein can also be found (Piascik et al., 1997). Liver contains predominantly α_{1B}-adrenoceptors in rats (Rokosh et al., 1994), but α_{1A}-adrenoceptors in humans (Price et al., 1994b). Most other tissues express mixtures of the three subtypes, and the relative expression levels have been found to be different in different reports.

The relationship between mRNA and protein expression is not always obvious. However, combined with radioligand binding and functional experiments, it is reasonable to conclude that most tissues express mixtures of α_1-adrenoceptor subtypes. These subtypes appear to coexist in different densities and ratios, and in most cases responses to α_1-adrenoceptor selective agonists are probably due to activation of more than one subtype. Tissues which have been used as model systems to characterize the pharmacological and signaling properties of particular subtypes (α_{1A}: rat vas deferens, rat submaxillary gland, rat kidney; α_{1B}: rat spleen, rat liver; α_{1D}: rat aorta) most likely express multiple subtypes, which may contribute to the confusion surrounding the properties of the native subtypes. The exception appears to be rat liver, which appears to express essentially only the α_{1B} subtype (Price et al., 1994a; Rokosh et al., 1994; Scofield et al., 1995).

Whether this tissue heterogeneity is due to expression of multiple cell types each expressing a single subtype, or coexpression of subtypes by cells is not yet completely clear. Analysis of α_1-adrenoceptor expression in clonal cell lines shows that most cell lines expressing α_1-adrenoceptors express only a single subtype, the α_{1B} (Han et al., 1992; Zhong and Minneman, 1999b). There are two exceptions, human SKNMC neuroepithelioma cells which express all three subtypes (Esbenshade et al., 1993; Price et al., 1994a) and rat medullary thyroid carcinoma (rMTC) 6-23 thyroid cells, which express mainly α_{1B} but detectable α_{1D} mRNA (Esbenshade et al., 1994). Expression of all three subtypes has also been found in primary cultures of rat neonatal cardiomyocytes, where they are differentially regulated by chronic agonist exposure and other hypertrophic stimuli (Rokosh et al., 1996). This supports the concept of coexpression in single cells, with expression levels of different subtypes subject to independent regulation.

5. Selective agonists and antagonists

The affinities and selectivities of drugs for α_1-adrenoceptor subtypes have been determined primarily by competition for radioligand binding to heterologously expressed recombinant subtypes. Most antagonists, including the prototype α_1-adrenoceptor selective antagonist prazosin, show little or no selectivity between the three known α_1-adrenoceptor subtypes (Hancock, 1996), consistent with the structural homology of these subtypes in the transmembrane domains (Fig. 2). However, a variety of drugs with varying degrees of selectivity have been identified.

Development of α_{1A}-adrenoceptor selective antagonists has been most successful. The first α_{1A}-adrenoceptor selective antagonist to be identified was WB 4101 (Morrow and Creese, 1986), which has about a 20-fold higher affinity for the α_{1A} than α_{1B} subtype. This limited selectivity, combined with an intermediate affinity for the α_{1D} subtype, limits its utility in differentiating between subtypes. Subsequently, 5-methylurapidil (Gross et al., 1988) and (+)-niguldipine (Boer et al., 1989) were identified as α_{1A}-adrenoceptor selective antagonists. These drugs had a greater selectivity between α_{1A} and α_{1B}-adrenoceptors (80–500-fold) and a low affinity for the α_{1D} subtype, making them useful for distinguishing subtypes in radioligand binding assays (Han and Minneman, 1991). However, each of these drugs had other properties that limited their use in functional experiments. 5-Methylurapidil is a partial agonist at serotonin 5-HT_{1A} receptors, while (+)-niguldipine is a dihydropyridine Ca^{2+} channel antagonist. A derivative of (+)-niguldipine, SNAP 5089 (2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid-*N*-[3-(4,4-diphenylpiperidin-1-yl)propyl]amide), was developed which is highly α_{1A}-selective but has a much lower affinity for voltage-gated Ca^{2+} channels (Wetzel et al., 1995). Additional α_{1A}-adrenoceptor selective antagonists which have been identified include KMD-

3213 ((−)-(*R*)-1-(3-hydroxypropyl)-5-[2-[2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethylamino]propyl] indoline-7-carboxamide; Shibata et al., 1995); RS 17053 (*N*-[2-(2cyclopropylmethoxyphenoxy)ethyl]5-chloro-α,α-dimethyl-1*H*-indole3-ethylamine hydrochloride; Ford et al., 1996); Rec 15/2739 (*N*-[3-[4-(2-methoxyphenyl)-1-piperazinyl] propyl]-3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxamide; Leonardi et al., 1997), A131701 (3-[2-((3a*R*,9b*R*)-*cis*-6-methoxy-2,3,3a,4,5,9b, hexahydro-[1*H*]-benz[*e*]isoindol-2-yl)ethyl]pyrido [3′,4′:4,5] thieno[3,2-d]pyrimidine-2,4(1*H*,3*H*)-dione; Hancock et al., 1998) and others (Hancock, 1996; Nagarathnam et al., 1998; Patane et al., 1998). These drugs generally have a relatively low affinity for both the α_{1B} and α_{1D} subtypes. Interest in this area has been strong, since α_{1A}-adrenoceptor selective antagonists may have significant therapeutic advantages over non-subtype selective α_1-adrenoceptor antagonists in treatment of benign prostatic hypertrophy (Nagarathnam et al., 1998). The moderately α_{1A}-adrenoceptor selective antagonist tamsulosin has been introduced for this purpose (Foglar et al., 1995).

Finding selective antagonists for α_{1B} and α_{1D}-adrenoceptors has been more difficult. Chlorethylclonidine was originally identified as an α_{1B}-adrenoceptor selective alkylating agent (Han et al., 1987a), however, complications in the use of site-directed alkylating agents and controversies over its selectivity in inactivating the recombinant α_1-adrenoceptor subtypes (Michel et al., 1993; Hirasawa et al., 1997) have pushed it into disfavor. Some competitive antagonists have been reported to be moderately α_{1B}-adrenoceptor selective, including spiperone and cyclazosin (Hancock, 1996), however the very limited selectivity of these agents has essentially precluded their use in subclassifying receptors. On the other hand, the partial serotonin $5HT_{1A}$ receptor agonist BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride) has been shown to be a selective antagonist at the α_{1D} subtype, with about 100-fold higher affinity for the α_{1D} than the α_{1A} or α_{1B} subtype (Goetz et al., 1995). This drug has proven useful in clarifying functional roles for the α_{1D} subtype (Piascik et al., 1995).

Comparing the affinities of agonists for receptors is much more difficult than comparing the affinities of antagonists because of the problems in determining agonist affinities in both radioligand binding and functional studies. However, some information is available on the comparative potencies and efficacies of agonists at the cloned α_1-adrenoceptor subtypes. The partial agonist oxymetazoline shows selectivity in radioligand binding assays, with about a 20-fold higher apparent affinity for the α_{1A} than α_{1B} or α_{1D} subtypes (Minneman et al., 1988; Hancock, 1996). Oxymetazoline and methoxamine also appear to selectively activate responses mediated by the α_{1A} over α_{1B} subtypes (Tsujimoto et al., 1989). Immediately following the initial cloning of the three subtypes it was noted that catecholamines and other agonists showed a 20-fold higher apparent affinity for the α_{1D} subtype than either the α_{1A} or α_{1B} (Lomasney et al., 1991; Perez et al., 1991).

A systematic examination of the potencies of a variety of full and partial agonists at the recombinant subtypes was performed by Minneman et al. (1994). Radioligand binding studies confirmed that full agonists such as adrenaline and noradrenaline showed about a 20-fold higher apparent affinity for the α_{1D} subtype compared to the other two, and that this was unaffected by experimental conditions such as the presence or absence of nucleotides and cations. Surprisingly, however, this potency difference was not apparent when receptor-mediated second messenger responses were measured in intact cells. Noradrenaline, adrenaline, 6-fluoronoradrenaline and phenylephrine had similar potencies and apparent intrinsic activities for stimulating inositol phosphate formation mediated by recombinant α_{1A}, α_{1B}, and α_{1D} subtypes in human embryonic kidney (HEK) 293 cells (Minneman et al., 1994). Methoxamine was most potent at the α_{1A} subtype but had a significant intrinsic activity at all three subtypes, while a variety of imidazolines, including cirazoline, showed a significantly higher intrinsic activity at the α_{1A} subtype compared to the other two. The selectivity of oxymetazoline and cirazoline for the recombinant α_{1A} subtype was also reported by Horie et al. (1995a,b). Other α_{1A}-adrenoceptor selective agonists have also been reported, including SDZ NVI 085 (3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2*H*-naphth [2,3-b]-1,4-oxazine hydrochloride; Hancock, 1996) and A61603 (*N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydro naphthalen-1-yl] methanesulfonamide hydrobromide; Knepper et al., 1995). These studies suggested that noradrenaline and adrenaline activate all three α_1-adrenoceptor subtypes with similar potencies, but that many synthetic agonists show significant selectivity between subtypes.

There is a clear need for additional selective agonists and antagonists, particularly for the α_{1B} and α_{1D} subtypes. Such compounds would be very useful in allowing quantitative evaluation of the distribution of subtypes by radioligand binding assays, and in defining their functional roles. However, the lack of clearly defined potential therapeutic applications for such compounds reduces the likelihood of their identification.

6. Structural determinants of selectivity

The role of particular domains and/or amino acid residues of the receptors in determining their drug specificities have begun to be elucidated. The aspartate in the third transmembrane domain and the two serines in the fifth transmembrane domain that are conserved in all catecholamine receptors probably interact with the protonated amine and two hydroxyls of the catecholamines, in a manner analogous to that reported for the β_2-adrenoceptor.

However, there is a significant difference in orientation of the catechol ring in the binding pocket due to a further separation of the serines in the α_1-adrenoceptors (Hwa and Perez, 1996).

Using oxymetazoline, cirazoline and methoxamine as α_{1A}-adrenoceptor selective agonists, Hwa et al. (1995) used site-directed mutagenesis to identify critical residues in α_{1A} and α_{1B}-adrenoceptors responsible for apparent differences in agonist binding potency. They found that conversion of alanine^{204} to valine in the fifth transmembrane domain and leucine^{314} to methionine in the sixth transmembrane domain of the α_{1B} subtype increased the affinity of these selective agonists until they were similar to their affinities for the α_{1A} subtype. The reciprocal double mutant in the α_{1A}-adrenoceptor decreased the affinities of these agonists so that they were similar to the α_{1B}. Hwa et al. (1995) developed a model suggesting that these two residues are critically involved in subtype-selective agonist binding, and may interact structurally within the receptors.

Similar studies with antagonists and the use of α_{1A}/α_{1B} chimeras allowed identification of residues involved in subtype-selective antagonist binding (Zhao et al., 1996). This study suggested that the fifth transmembrane domain and a portion of the second extracellular loop are critically important in subtype-selective antagonist binding. In particular, three adjacent residues located on the extracellular loop of the fifth transmembrane domain appeared to be fully responsible for the higher antagonist affinity for α_{1A}-adrenoceptors. This suggests that α_1-adrenoceptor antagonists may bind near the surface of the receptor, rather than deep within the transmembrane domains like the agonists. Involvement of the second transmembrane domain in specifying the selectivity of niguldipine derivatives between the α_{1A} and α_{1D} subtypes was also reported by Hamaguchi et al. (1996).

Other than these studies, little is known about the molecular determinants of the pharmacological differences between α_1-adrenoceptor subtypes. Additional information would clearly be useful in helping to predict structures of additional subtype-selective drugs.

7. G protein coupling

α_1-Adrenoceptors belong to the larger family of $G_{q/11}$-coupled G protein coupled receptors, which initiate signals by activating phospholipase C-dependent hydrolysis of phosphatidylinositol 4,5, bisphosphate. This enzyme generates the second messengers inositol (1,4,5) trisphosphate, which releases Ca^{2+} from intracellular stores, and diacylglycerol, which synergizes with Ca^{2+} to activate protein kinase C (Minneman, 1988; Hieble et al., 1995; Fig. 3). The $G_{q/11}$ family of G proteins contains four α subunits, α_q and α_{11} which are coexpressed in most cells, and α_{14} and α_{16} which show a much more restricted distribution.

Coupling of individual α_1-adrenoceptor subtypes to different α subunits of the $G_{q/11}$ family has been examined by transient overexpression of both receptor and G protein α subunits in monkey kidney COS-7 cells. These studies showed that all three α_1-adrenoceptor subtypes could couple to phospholipase C through α_q and α_{11}, only α_{1A} and α_{1B} subtypes coupled to α_{14}, and only α_{1B} coupled to α_{16} (Wu et al., 1992). Coupling of all three α_1-adrenoceptor subtypes to both G_q and G_{11} is supported by studies from Wise et al. (1995). These investigators showed that agonist treatment of rat 1 fibroblasts expressing α_{1A}, α_{1B} or α_{1D}-adrenoceptors accelerated degradation of both α_q and α_{11}. These studies suggest that individual receptors can activate multiple G protein α subunits, but also support some specificity in G protein activation.

The reciprocal knockout experiments have been reported recently with surprising results. Xu et al. (1998) examined α_1-adrenoceptor mediated responses in pancreatic and submandibular gland cells from mice genetically deficient in individual G protein α subunits. These cells normally express three of the four $G_{q/11}$ α subunits (α_q, α_{11}, and α_{14}). Surprisingly, knockout of each of these α subunits individually had no measurable effect on Ca^{2+} responses to adrenaline in these cells, or on signals generated by activation of other receptors acting through this G protein family. The authors interpreted these results to suggest that receptors promiscuously activate all $G_{q/11}$ family members, and if one of these is removed the remaining family members can fully compensate for its absence. More recent studies have suggested that signaling specificity may be conferred by interaction with RGS (Regulators of G Protein Signaling) proteins (Xu et al., 1999).

The use of antisense oligonucleotides to address the importance of particular G protein α subunits in α_1-adrenoceptor mediated responses has suggested the contradictory hypothesis that α_q and α_{11} mediate distinct functions. Using myocytes from rat portal vein, Macrez-Lepretre et al. (1997) showed that antisense knockout of α_q but not α_{11} reduced the Ca^{2+} response in the absence of extracellular Ca^{2+}, while in the presence of extracellular Ca^{2+} antisense knockout of α_{11} reduced store-operated Ca^{2+} influx. This suggests different functional roles for α_q and α_{11}. The difference between the genetic and antisense knockout experiments is not yet clear, although it may be a result of phenotypic differences between cells, differences in the α_1-adrenoceptor subtype(s) involved, and/or the influence of RGS proteins (Xu et al., 1999).

The domains of the α_{1B}-adrenoceptor involved in coupling to $G_{q/11}$-mediated phospholipase C activation were first studied by Cotecchia et al. (1990). Using chimeric α_{1B}/β_2-adrenoceptor constructs, these investigators demonstrated the critical importance of the third cytoplasmic loop in coupling to inositol phosphate formation, and the relative unimportance of the C-terminal tail. This is

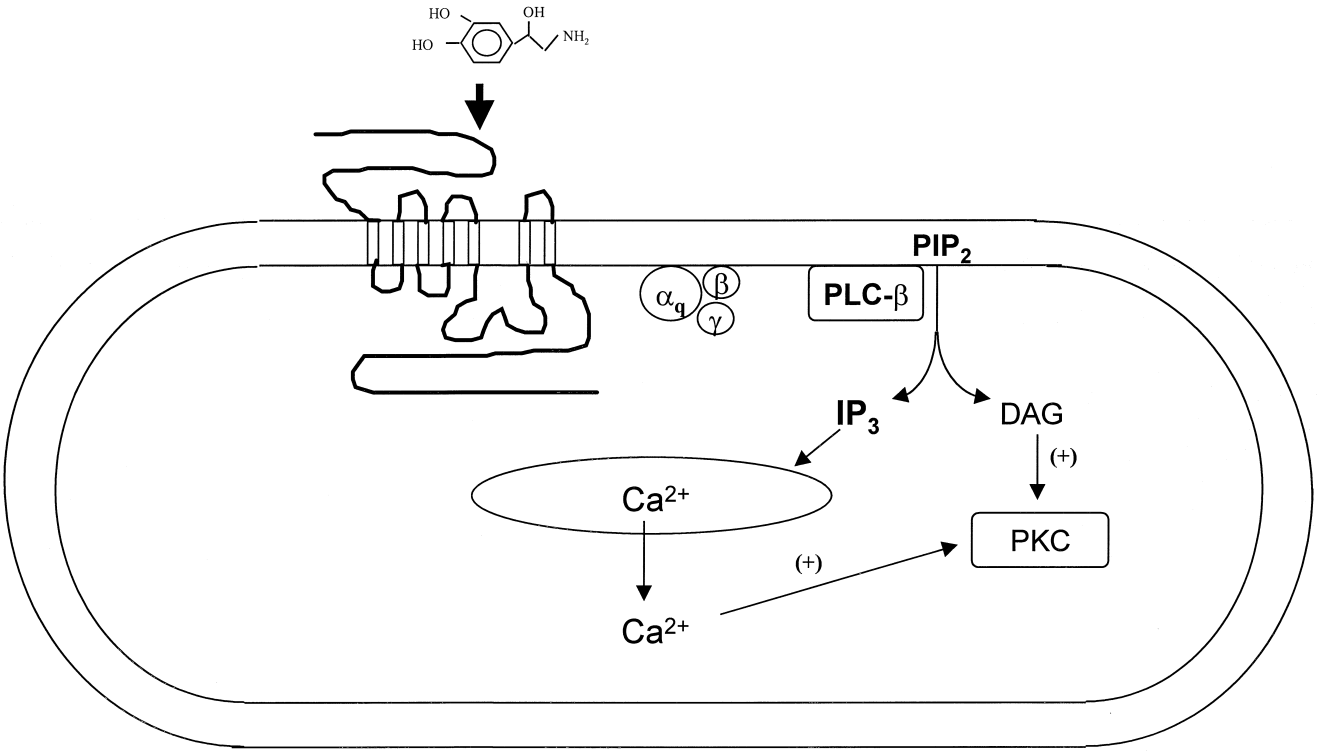

Fig. 3. Traditional signaling pathways activated by α_1-adrenoceptor subtypes. Binding of noradrenaline to the membrane spanning receptor is known to activate the $G_{q/11}$ family of heterotrimeric G proteins. The α and β/γ subunits dissociate and activate phospholipase C (PLC)-beta. This enzyme hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) into two products, inositol(1,4,5)trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to receptors on the endoplasmic reticulum to release stored intracellular Ca^{2+}. This released Ca^{2+} acts synergistically with DAG to activate protein kinase C (PKC), which phosphorylates specific target proteins in the cell to change their functions.

similar to results with many other G protein coupled receptors, and is supported by studies showing that overexpression of the third cytoplasmic loop of the α_{1B}-adrenoceptor inhibits coupling of this receptor, but not other receptors, to inositol phosphate formation (Luttrell et al., 1993).

Recombinant α_{1B}-adrenoceptors have also been reported to couple to other G proteins, particularly $G\alpha_o$ (Blitzer et al., 1993) and $G\alpha_S$ (Horie et al., 1995a,b) following overexpression, although the functional relevance of this coupling is not yet clear. Studies with specific G protein antibodies have supported the hypothesis that native α_{1B} (but not α_{1A} or α_{1D})-adrenoceptors can also couple to $G\alpha_o$ in rat aortic smooth muscle (Gurdal et al., 1997), suggesting a functional role for this coupling. α_1-Adenoceptors have also been shown to activate a high molecular weight G protein G_h, which is transglutaminase II (Nakaoka et al., 1994). It has been reported that activation of G_h occurs only by transfected α_{1B} and α_{1D} subtypes, and not α_{1A} (Chen et al., 1996), and is independent of its transglutaminase activity. However, the role of G_h in cellular responses to catecholamines is still obscure.

8. Coupling to other second messenger systems

A variety of other signaling pathways have also been shown to be activated by α_1-adrenoceptors (Minneman, 1988). These include Ca^{2+} influx, arachidonic acid release, and phospholipase D activation. Shortly after the existence of pharmacologically distinct α_1-adrenoceptor subtypes was recognized, Han et al. (1987b) suggested that the α_{1A} subtype coupled selectively to voltage-gated Ca^{2+} influx in smooth muscle. This hypothesis was supported by some studies (Tsujimoto et al., 1989; Han et al., 1990; Suzuki et al., 1990; Han and Minneman, 1991), but not others (Sulpizio and Hieble, 1991), and it now seems clear that such responses are not specific to a single α_1-adrenoceptor subtype.

The mechanisms by which Ca^{2+} influx is increased following α_1-adrenoceptor activation are not always completely clear. As with most $G_{q/11}$ linked receptors, activation of α_1-adrenoceptors often activates a capacitative Ca^{2+} influx which is not voltage-gated and not blocked by dihydropyridine Ca^{2+} channel blockers (Minneman, 1988). In rat portal vein myocytes, for example, α_1-adrenoceptors cause Ca^{2+}-activated Ca^{2+} influx that replenishes intracellular Ca^{2+} stores through a mechanism involving α_{11} (Macrez-Lepretre et al., 1997). Similarly, activation of α_1-adrenoceptors in rat aorta also activates capacitative Ca^{2+} entry subsequent to the initial increase in intracellular Ca^{2+} (Noguera et al., 1997).

However, α_1-adrenoceptor activation also increases dihydropyridine-sensitive Ca^{2+} influx (presumably through voltage-gated channels) in some tissues but not others.

Zhang et al. (1998) showed that activation of α_1-adrenoceptors potentiated L-type Ca^{2+} currents in rat ventricular myocytes in a protein kinase C dependent manner, suggesting that such effects may be secondary to increases in intracellular Ca^{2+} and activation of protein kinase C. However, α_1-adrenoceptors may also couple directly to activation of Ca^{2+} channels in some cells. In rat medullary thyroid carcinoma 6-23 cells, activation of α_{1B}-like adrenoceptors increased dihydropyridine-sensitive Ca^{2+} influx in a manner independent of release of intracellular Ca^{2+}, activation of phospholipase C and/or activation of protein kinase C (Esbenshade et al., 1994). Since this increase in intracellular Ca^{2+} was blocked by removal of extracellular Ca^{2+}, and occurred even in presence of thapsigargin, it suggested that there might be a direct coupling between these receptors and voltage-dependent Ca^{2+} influx.

Other signaling pathways have also been shown to be activated by α_1-adrenoceptors. Burch et al. (1986) showed that stimulation of α_1-adrenoceptors in a rat thyroid cell line increased release of arachidonic acid, which in turn mediated a noradrenaline-stimulated cell replication. Other studies have shown that stimulation of α_1-adrenoceptors can cause arachidonic acid release in many cells (Kanterman et al., 1990; Blue et al., 1994), either through activation of phospholipase A_2 (Kanterman et al., 1990; Nishio et al., 1996; Xing and Insel, 1996) or phospholipase D (Ruan et al., 1998). Some evidence suggests that such increases in arachidonic acid release require extracellular Ca^{2+} but are independent of increases in intracellular Ca^{2+} (Kanterman et al., 1990), suggesting a role for phospholipase A_2. Studies in rabbit aortic smooth muscle cells support the involvement of a pertussis toxin sensitive G protein (Nishio et al., 1996), while other studies provide evidence for the involvement of protein kinase C or mitogen activated protein kinase pathways. Perez et al. (1993) directly compared activation of arachidonic acid release by α_{1B}- and α_{1D}-adrenoceptors after overexpression in two different cell lines. Their studies found that both subtypes can cause this response in both cell lines, but by different mechanisms. In COS-1 cells, arachidonic acid release was mediated through phospholipase A_2 activation, involved a pertussis toxin-sensitive G protein, and required Ca^{2+} influx through dihydropyridine-sensitive channels. In CHO cells, however, slightly different mechanisms were observed. Even though CHO cells lack voltage-sensitive Ca^{2+} channels, both subtypes were still able to activate arachidonic acid release. Therefore α_{1B}- and α_{1D}-adrenoceptor subtypes can couple to phospholipase A_2 activation via a pertussis toxin-sensitive pathway in CHO cells, and a single α_1-adrenoceptor subtype can activate arachidonic acid release through different mechanisms in different cells.

More recently, activation of arachidonic acid release has been shown to be downstream of mitogen activated protein kinase pathways. In Madin-Darby canine kidney cells, immunoprecipitation studies combined with specific inhibitors showed that an 85-kD cytosolic phospholipase A_2 was activated by α_1-adrenoceptor stimulation. This activation was blocked by PD 98059, a specific inhibitor of mitogen activated protein kinase pathways, which also inhibited adrenaline-promoted arachidonic acid release. Down-regulation or inhibition of protein kinase C also blocked mitogen activated protein kinase activation and arachidonic acid release. Therefore, α_1-adrenoceptors appear to regulate arachidonic acid release through phosphorylation-dependent activation of phospholipase A_2 by mitogen activated protein kinases, subsequent to activation of protein kinase C in these cells (Xing and Insel, 1996). Whether this represents a general mechanism for α_1-adrenoceptor activation of phospholipase A_2 remains to be tested.

Activation of phospholipase D by α_1-adrenoceptor subtypes has also been studied (Llahi et al., 1992; Balboa and Insel, 1998, Ruan et al., 1998). Although the mechanisms involved in this activation are poorly understood, a role for protein kinase C has been proposed. Balboa and Insel (1998) studied activation of phospholipase D stimulated by adrenaline in Madin-Darby canine kidney cells. They found that although adrenaline stimulated protein kinase Cα and protein kinase Cε, this was not associated with activation of phospholipase D. Furthermore, blocking phospholipase C activation with neomycin did not significantly decrease adrenaline-stimulated phospholipase D activity. Chelation of extracellular Ca^{2+} markedly inhibited phospholipase D activation, suggesting a role for Ca^{2+} in phospholipase D activation by α_1-adrenoceptors. Ruan et al. (1998) studied α_1-adrenoceptor-mediated stimulation of arachidonic acid release by activation of phospholipase D in Rat-1 fibroblasts. After overexpression, α_{1A}-adrenoceptors increased arachidonic acid release, cAMP levels, and phospholipase D activity. The increase in arachidonic acid release was attenuated by inhibition of phospholipase D, and by increases in cyclic AMP caused by forskolin or addition of a non-hydrolyzable analog of cAMP. They also showed that all three subtypes of adrenoceptors were able to activate phospholipase D in these cells, although to different extents ($\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$). Therefore, all three α_1-adrenoceptor subtypes appear to be able to activate phospholipase D, although the mechanisms may differ in different cell types.

9. Mitogenic responses

Mitogenic responses have traditionally been thought to be activated primarily by peptide growth factors, such as epidermal growth factor and nerve growth factor. Growth factor receptors consist of single polypeptide chains containing a single transmembrane domain. These receptors have intrinsic tyrosine kinase activity and dimerize in response to agonist occupation. Mitogenic responses are caused by a conserved cascade of events including recep-

tor phosphorylation, binding of adaptor proteins, such as Shc and Grb2, and eventually recruitment and activation of the small molecular weight G protein Ras. Ras then activates Raf, which activates downstream mitogen activated protein kinase cascades (reviewed in Davis, 1993). It is now clear that G-protein coupled receptors can rapidly activate mitogen-activated protein kinase pathways, and this activation plays an important role in regulation of cell proliferation and growth. Despite intensive study, the mechanisms underlying the mitogenic effects of G protein coupled receptor activation are still not well understood.

G protein coupled receptors acting through G_s, G_i, and $G_{q/11}$, have all been shown to activate mitogen activated protein kinase pathways (Howe and Marshall, 1993; Winitz et al., 1993; Crespo et al., 1995; Lev et al., 1995; Van Bliesen et al., 1995; Dikic et al., 1996), although the mechanisms involved appear to be dependent on cell phenotype (Fig. 4). In some cases, activation of these pathways is downstream of known second messengers such as cyclic AMP (Crespo et al., 1995), Ca^{2+} (Lev et al., 1995; Dikic et al., 1996), and/or protein kinase C (Della Rocca et al., 1997). In other cells, however, α and/or βγ subunits may directly or indirectly activate the Ras/Raf pathway through adapter proteins, tyrosine kinases, and/or phosphoinositide-3-kinase (Lopez-Ilasaca et al., 1997). Activation of these pathways by G_i coupled receptors may require the βγ subunits of G proteins, since sequestration of βγ subunits often inhibits mitogen activated protein kinase activation (Yamauchi et al., 1997). For G_q coupled receptors, however, the mechanisms are not yet clear. Both α_q and βγ mediated activation of mitogen activated protein kinases have been proposed.

α_1-Adrenoceptors, like other G-protein coupled receptors, activate mitogenic responses in a variety of cell types and play important roles in regulating cell growth and proliferation. α_1-Adrenoceptor agonists stimulate hypertrophy in cultured neonatal rat ventricular myocytes (LaMorte et al., 1994), and overexpression of a constitutively active mutant of the α_{1B}-adrenoceptor in cardiac myocytes induces myocardial hypertrophy in transgenic animals (Milano et al., 1994). Other studies have shown that α_1-adrenoceptors can activate various mitogen activated protein kinase pathways in many cells and tissues, and may play an important role in regulating cell growth

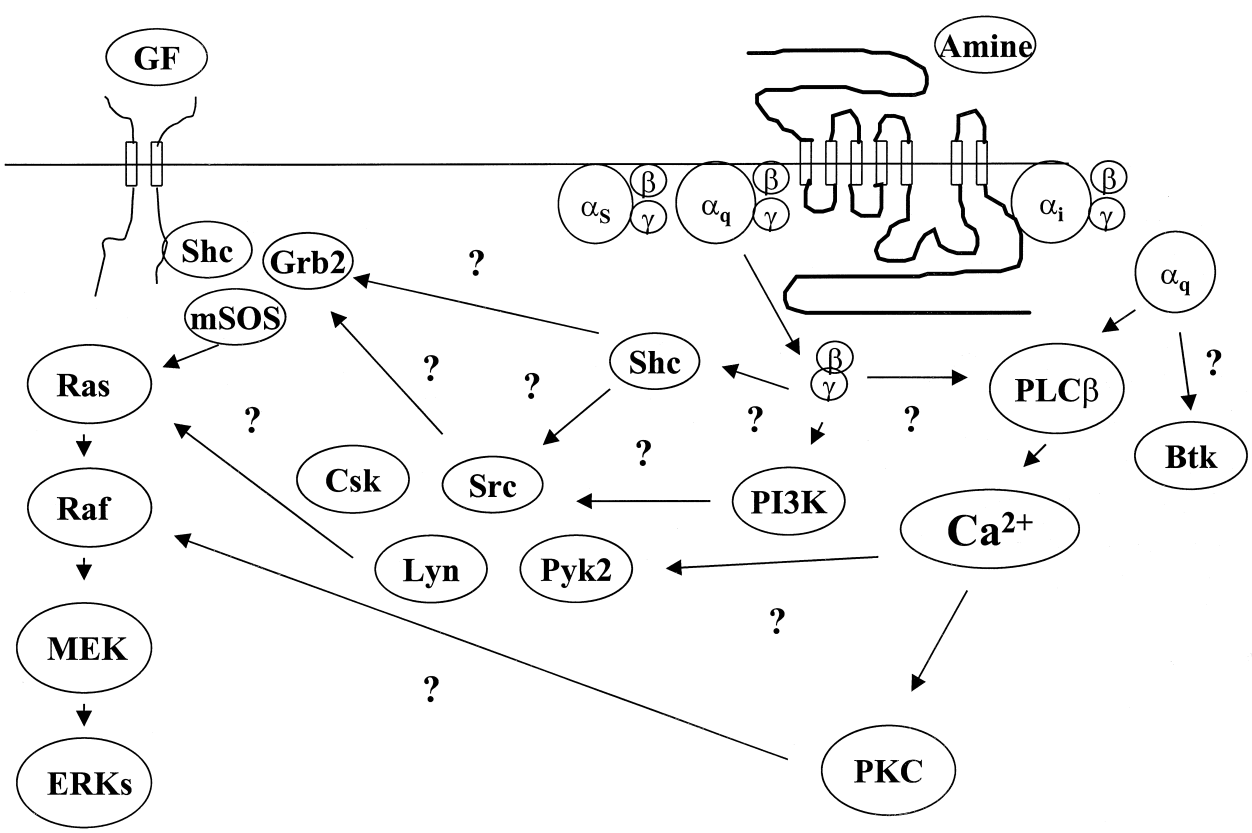

Fig. 4. Mitogenic signaling mechanisms activated by growth factor and G protein coupled receptors. Growth factors (GF) are known to activate various mitogen activated protein kinase (MAPK) pathways through activation of a cascade of tyrosine phosphorylation and protein–protein association. This involves the adapter proteins Shc, Grb2, and mSOS which associate to activate the small molecular weight G protein Ras. Ras activates a cascade of dual specificity kinases called Raf and MEK, ultimately activating the serine/threonine kinase ERKs, which phosphorylate specific transcription factors. The mechanisms by which biogenic amines act through G protein coupled receptors to activate these pathways are much less clear. There may be direct transactivation of the growth factor receptors, effects of various second messengers such as Ca^{2+} and protein kinase C (PKC), direct effects of various G protein subunits, or other mechanisms involved. They may involve various tyrosine kinases, including Pyk2, Csk, Src, Btk and/or Lyn; adapter proteins such as Shc; possibly with the involvement of phosphoinositide-3-kinase (PI3K). The mechanisms may be highly dependent on cell phenotype.

(Ramirez et al., 1997; Spector et al., 1997; Alexandrov et al., 1998; Lazou et al., 1998). Zechner et al. (1997) studied the role of α_1-adrenoceptor stimulated mitogen activated protein kinases in myocardial cell hypertrophy. Treatment of myocardial cells with the α_1-adrenoceptor agonist phenylephrine induced the hallmark features of cardiac cell hypertrophy: increases in cell size, sarcomeric organization, and induction of certain cardiac-specific genes. Phenylephrine, in the presence of propranolol to block β-adrenoceptors, also stimulated the dual-phosphorylation of p38 mitogen activated protein kinase, extracellular signal regulated protein kinase, and to a lesser extent, c-Jun-NH_2 terminal kinase. They further showed that a specific p38 inhibitor was able to block all the hypertrophic responses stimulated by phenylephrine, suggesting that α_1-adrenoceptors can affect cardiac cell hypertrophy by activation of p38 mitogen activated protein kinases.

Ramirez et al. (1997) also studied the effect of α_1-adrenoceptor activation on cardiac cell hypertrophy, specifically transcriptional activation of atrial natriuretic factor gene expression by α_1-adrenoceptor stimulation and overexpression of Ras. Treatment with phenylephrine activated extracellular signal regulated kinases and c-Jun-NH_2 terminal kinase, however overexpression of Ras only activated c-Jun-NH_2 terminal kinase and caused little increase in extracellular signal regulated kinase activity. This study suggests a pathway involving Ras and c-Jun-NH_2 terminal kinase is involved in α_1-adrenoceptor regulated atrial natriuretic factor gene expression. Spector et al. (1997) studied the role of α_1- and β_2-adrenoceptor stimulated mitogen activated protein kinases in quiescent and regenerating rat hepatocytes. α_1-Adrenoceptors activated all three arms of mitogen activated protein kinase pathway in quiescent cells. However, p38 mitogen activated protein kinase played a more important role than extracellular signal regulated kinases, since a specific p38 inhibitor effectively inhibited cell proliferation.

α_1-Adrenoceptors also regulate smooth muscle growth. Xin et al. (1997) studied the role of α_1-adrenoceptors in catecholamine regulation of smooth muscle cell growth. Noradrenaline, probably acting through α_{1D}-adrenoceptors, caused rapid and transient activation of extracellular signal regulated protein kinases and increased protein synthesis and cell size. These responses were inhibited by the selective inhibitor of extracellular signal regulated kinases PD 98059. These data suggest that smooth muscle cell growth induced by noradrenaline may be mediated by α_{1D}-adrenoceptors that couple to activation of extracelluar signal regulated kinase cascades.

In rat pheochromacytoma PC12 cells transfected with α_{1A}-adrenoceptors, noradrenaline also activates all three major arms of the mitogen activated protein kinase pathway. Noradrenaline treatment of α_{1A}-transfected PC12 cells activates extracellular signal regulated protein kinases, c-Jun-NH_2-terminal kinases, and p38 mitogen activated protein kinases (Williams et al., 1998). In α_{1A}-transfected PC12 cells, noradrenaline also causes cells to differentiate into a neuronal like phenotype similar to that caused by treatment with nerve growth factor.

It is clear that α_1-adrenoceptors can activate a variety of mitogen activated protein kinase pathways to regulate various cellular functions. The mechanisms involved in this regulation are likely to depend on the particular cellular environment and phenotype. The multiple pathways may also interact with each other in unexpected ways. Recently, Alexandrov et al. (1998) showed that activation of p38 mitogen activated protein kinase by α_1-adrenoceptors in Rat-1 cells had a negative effect on activation of extracellular signal regulated kinases. However, the significance of this regulation and its consequences need further study.

10. Involvement of second messengers in mitogenic responses

Not surprisingly, activation of extracellular signal regulated protein kinases by receptors coupled to pertussis toxin-sensitive G_i proteins and pertussis toxin-insensitive $G_{q/11}$ proteins appear to involve different mechanisms (Hawes et al., 1995). Receptors coupled to G_i, such as α_2-adrenoceptors, appear to activate extracellular signal regulated protein kinases through release of βγ subunits, since responses to these receptors are often blocked by coexpression of an intracellular sequestrant of free βγ subunits. In addition, transient expression of βγ, but not α_i, is often sufficient to induce Ras-dependent extracellular signal regulated protein kinase activation.

For $G_{q/11}$ coupled receptors, however, the mechanism of activation of mitogen activated protein kinase pathways is not completely clear. Some evidence supports the idea that both α and βγ subunits may be involved (Yamauchi et al., 1997). Other studies have examined the role of downstream second messengers in $G_{q/11}$ coupled receptor mediated mitogen activated protein kinase activation. Protein kinase C-dependent (Hawes et al., 1995), fully-protein kinase C independent (Charlesworth and Rozengurt, 1997) and partially protein kinase C-independent (Crespo et al., 1994) mechanisms have been reported.

Bogoyevitch et al. (1996) studied adrenoceptor stimulation of mitogen-activated protein kinase pathways in cardiac ventricular myocytes. These investigators showed that prolonged phorbol ester exposure partially down-regulated the activation of mitogen-activated protein kinases by noradrenaline but not by isoprenaline, implicating a role for protein kinase C in the α_1-adrenoceptor response. Unfortunately, the particular α_1-adrenoceptor subtype involved was not clearly identified. Romanelli and van de Werve (1997) investigated the role of protein kinase C in activation of mitogen-activated protein kinases in isolated rat hepatocytes. These authors showed that adrenaline stimu-

lated mitogen activated protein kinase activity was inhibited by pretreatment of hepatocytes with the protein kinase C inhibitor Ro-31-8220, suggesting the involvement of protein kinase C in this response.

We studied the role of Ca^{2+} and protein kinase C in α_1-adrenoceptor mediated activation of mitogen-activated protein kinase pathways in PC12 cells. In PC12 cells stably transfected with the human α_{1A}-adrenoceptor, noradrenaline strongly activated both extracellular signal regulated kinases and c-Jun-NH_2-terminal kinases. These responses were not mediated by increases in intracellular Ca^{2+}, since depletion or chelation of intracellular Ca^{2+} stores had no effect on these responses (Berts et al., 1999). In addition, inhibition of protein kinase C did not affect the α_1-adrenoceptor response, although it blocked similar responses to phorbol esters. These results suggest that Ca^{2+} release and protein kinase C activation are neither necessary nor sufficient for α_{1A}-adrenoceptor mediated activation of mitogenic responses in PC12 cells. Overall, therefore, it is clear that α_1-adrenoceptors can mediate activation of mitogen activated protein kinase pathways through both protein kinase C sensitive (Hawes et al., 1995) and insensitive pathways. It is likely that the mechanisms involved may be highly dependent on cell phenotype.

11. Activation of tyrosine kinases and small G proteins

Other than Ca^{2+} and protein kinase C, a number of studies have tried to identify other signaling molecules involved in activation of mitogen activated protein kinase pathways. It has been proposed that G_i coupled receptors activate mitogen activated protein kinase pathways through $\beta\gamma$ activation of Ras. $\beta\gamma$ dimers have been shown to be sufficient to induce Ras activation, as shown by the induced accumulation of Ras in the GTP-bound active form (Koch et al., 1994). Ras involvement in responses to $G_{q/11}$ coupled receptors is not yet completely clear. In neonatal rat ventricular myocytes, stimulation of α_1-adrenoceptors activates c-Jun NH_2 terminal kinase in a Ras-dependent manner, and this pathway is involved in cardiac hypertrophy (Ramirez et al., 1997). In the same study, however, a constitutively active Ras mutant was not able to increase extracellular signal regulated protein kinase activity. Therefore, Ras activation may be selectively involved in activation of c-Jun NH_2 terminal kinase but not extracellular signal regulated protein kinase.

LaMorte et al. (1994) studied the role of α_q and Ras in α_1-adrenoceptor stimulated atrial natriuretic factor gene expression. Although introduction of a constitutively active form of α_q was sufficient to induce expression of atrial natriuretic factor, coexpression of an inhibitory form of Ras along with a constitutively activated α_q did not inhibit atrial natriuretic factor expression stimulated by activated α_q. Studies by Della Rocca et al. (1997) examined the role of Ras in α_{1B}-adrenoceptor stimulated extracellular signal regulated protein kinase activation. Their results showed that this response was attenuated by coexpression of a dominant negative mutant of Ras, suggesting that Ras may participate in this pathway. Hu et al. (1999) studied the activation of Ras by α_{1A}- and α_{1B}-adrenoceptors in transfected NIH3T3 cells. Activation of both subtypes activated Ras and stimulated guanine nucleotide exchange. Overexpression of a dominant negative Ras mutant attenuated α_{1B}- but not α_{1A}-adrenoceptor mediated activation of phosphatidylinositol-3-kinase. Therefore, both receptor subtypes were able to activate Ras, but with divergent downstream effect.

Other molecules in the Ras superfamily, such as Rho, may also be involved in cellular responses to α_1-adrenoceptor activation. Sah et al. (1996) studied α_1-adrenoceptor-mediated hypertrophy in neonatal rat ventricular myocytes. Dominant negative inhibitors of Rho markedly attenuated atrial natriuretic factor reporter gene expression induced by phenylephrine. However, these inhibitors were not able to block the activation of extracellular signal regulated kinase by phenylephrine, or Ras induced atrial natriuretic factor gene activation. Since activated Rho and Ras produced a large synergistic effect on atrial natriuretic factor-luciferase gene expression, they concluded that Rho functions in a pathway separate from Ras. Their results suggest that Rho is an effector of α_q signaling, and may be involved in α_1-adrenoceptor mediated regulation of cell growth.

Recent studies have suggested a role for non-receptor tyrosine kinases in $G_{q/11}$ coupled receptor mediated mitogenic responses. Tyrosine kinase inhibitors can block mitogenic responses to α_1-adrenoceptors in some preparations (Thorburn and Thorburn, 1994). The tyrosine kinase Pyk2 was originally cloned as a focal adhesion kinase related tyrosine kinase, and can be activated by $G_{q/11}$ coupled receptors in both protein kinase C sensitive and insensitive manners (Lev et al., 1995). Dikic et al. (1996) showed that activation of bradykinin receptors induced tyrosine phosphorylation of Pyk2 and complex formation between Pyk2 and activated Src. Tyrosine phosphorylation of Pyk2 leads to binding of the SH2 domain of Src, and therefore activation of Src. Overexpression of a dominant interfering mutant of Pyk2 or a negative regulator of Src kinase activity (Csk) inhibited activation of Pyk2 by G protein coupled receptors. Therefore, it was proposed that Pyk2 acts with Src to link $G_{q/11}$-coupled receptors to MAPK activation.

Della Rocca et al. (1997) examined the activation of extracellular signal regulated protein kinases by α_{1B}-adrenoceptors in human embryonic kidney 293 cells. α_{1B}-adrenoceptor mediated activation of extracellular signal regulated protein kinases was attenuated by expression of a dominant-negative mutant of Pyk2, a dominant negative mutant of Src, or by a negative regulator of Src, Csk. These studies established a role for Pyk2 and Src in α_1-adrenoceptor stimulated mitogenic responses. We stud-

ied activation of Pyk2 by α_{1A}-adrenoceptors in transfected PC12 cells. In these cells, noradrenaline strongly stimulated phosphorylation of Pyk2, however, this response was not dependent on increases in intracellular Ca^{2+}. Therefore, it is clear that α_1-adrenoceptors activate non-receptor tyrosine kinases in a variety of cell lines. However, how receptor activation is linked to tyrosine kinase activation and mitogenic responses remains to be clarified. Different sets of converging or parallel pathways may be activated in different cell types, and the final response may depend on integration of multiple signals.

12. Relative coupling efficiencies

It is clear that all three α_1-adrenoceptor subtypes couple to phospholipase C through the $G_{q/11}$ family to increase intracellular Ca^{2+}. However, the three cloned subtypes have been found to have different efficiencies in activating this pathway. Schwinn et al. (1991) first reported that the α_{1A}-adrenoceptor was more efficient than the α_{1B}-adrenoceptor in activating inositol phosphate formation. Perez et al. (1993) studied the coupling of expressed α_{1B}- and α_{1D}-adrenoceptors to different signaling pathways in different cell lines. Their studies showed that both α_{1D}- and α_{1B}-subtypes coupled to phosphoinositide hydrolysis through a pertussis toxin-insensitive G protein, although the coupling efficiencies of the two subtypes were not directly compared.

A systematic comparison of recombinant human α_1-adrenoceptors expressed at similar densities in human embryonic kidney 293 cells found that agonist-occupied α_1-adrenoceptor subtypes showed substantial differences in their ability to increase both inositol phosphate accumulation and intracellular Ca^{2+} ($\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$; Theroux et al., 1996). Similar results were obtained in human SKNMC neuroepithelioma cells, where different maximal responses were obtained following activation of each subtype, both for inositol phosphate formation ($\alpha_{1A} > \alpha_{1B} \geq \alpha_{1D}$) and Ca^{2+} release ($\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$). Taguchi et al. (1998) also compared the coupling of α_1-adrenoceptor subtypes to these responses, and examined activation of protein kinase C in rat-1 fibroblasts. A similar order of coupling efficiency was observed for the three subtypes for inositol phosphate formation ($\alpha_{1A} \geq \alpha_{1B} > \alpha_{1D}$) and Ca^{2+} ($\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$) responses, although the receptors were expressed at higher and variable densities. They also found that human α_{1A}- and α_{1B}-adrenoceptors activated protein kinase C α, δ, and ε, while α_{1D}-adrenoceptors did not give consistent protein kinase C activation.

Different subtypes of α_1-adrenoceptors may play different roles in controlling growth, differentiation, and cell fate through selective activation of one or more mitogen activated protein kinase pathways. Chen et al. (1995) studied the regulation of vascular smooth muscle growth by adrenoceptor subtypes. Although all three subtypes of adrenoceptors are expressed in these cells, only the α_{1B} subtype appeared to be involved in smooth muscle cell growth. Blocking the other subtypes actually potentiated the response. These authors proposed that prolonged stimulation of α_{1B}-adrenoceptors induces hypertrophy of arterial smooth muscle cells, and other adrenoceptors attenuate this response.

Other studies have compared the efficiencies of the three α_1-adrenoceptor subtypes in activating mitogenic responses. Siwik and Brown (1996) studied the regulation of protein synthesis by α_1-adrenoceptor subtypes in cultured rabbit aortic vascular smooth muscle cells. Competition binding studies with subtype selective antagonists showed that these cells express mainly α_{1B}-adrenoceptors (75%) relative to α_{1A}-adrenoceptors (25%). These subtypes increased protein synthesis in proportion to their relative abundance. Therefore, α_{1B}-adrenoceptors predominated in coupling to metabolic responses, in contrast to reports that contractile responses in the same tissue are preferentially mediated by α_{1A}-adrenoceptors. Wenham et al. (1997) studied the coupling of three α_1-adrenoceptor subtypes in cultured neonatal rat cardiac myocytes. Although mRNA transcripts for the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes were detected, binding sites for only the α_{1A}- and α_{1B}-adrenoreceptors were detected. This study showed that phenylephrine-induced activation of mitogen activated protein kinase cascades appeared to be mediated by a subtype resembling most closely the pharmacological profile of the α_{1B}. These studies support the idea that mitogenic responses may be caused by multiple α_1-adrenoceptor subtypes.

We directly compared the coupling of α_1-adrenoceptor subtypes to mitogenic responses in PC12 cells. We expressed each subtype under control of an inducible promoter, and compared their coupling to common second messenger responses as well as their ability to activate various mitogen activated protein kinase pathways (Zhong and Minneman, 1999a). At similar receptor densities, coupling to inositol phosphate formation and intracellular Ca^{2+} was similar to that observed in other cell lines ($\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$). For mitogenic responses, the α_{1A}-subtype was able to strongly activate all three arms of the pathway (extracellular signal regulated kinases, c-jun-NH_4-terminal activated kinases, and p38 mitogen activated protein kinases), the α_{1B} was weaker in stimulating these responses, and the α_{1D} only weakly activated extracellular signal regulated kinases. These differences were not due to subclone variation since nerve growth factor gave consistent responses in all subclones tested. Therefore, clear differences exist among the three adrenoceptor subtypes in their ability to activate various mitogenic responses, although the functional significance of this is not yet known.

13. Potential protein binding partners

Recently it has become clear that G protein coupled receptors can interact directly with proteins other than G

proteins. It is becoming increasingly likely that these proteins form macromolecular signaling complexes similar to, but different from those formed by growth factor receptors. Discrete sequences on the C-terminal tails of a number of receptors have been shown to bind directly to proteins containing PDZ domains, and interaction with other types of proteins have also been reported. This has been best studied with the β_2-adrenoceptor, which was recently reported to bind directly to the sodium/hydrogen exchanger regulatory factor via a C-terminal motif (Hall et al., 1997, 1998). Agonist activation of the receptor causes release of this factor, modulating sodium/hydrogen exchange. The β_2-adrenoceptor is phosphorylated on a tyrosine residue in the C-terminal tail to form an SH2 domain, which then interacts with the adapter molecule Grb2 (Shih and Malbon, 1998). β_2-Adrenoceptors have also been shown to form a complex with phosphatases, kinases and anchoring proteins (Shih et al., 1999), and it is not yet clear exactly how many proteins interact directly with the receptor.

No such interactions have yet been reported for α_1-adrenoceptor subtypes. However, it is intriguing that the consensus motif for the binding of the immediate-early gene Homer to metabotropic glutamate receptors is found in the C-terminal tail of the α_{1D}-adrenoceptor, but not the α_{1A} or α_{1B} (Tu et al., 1998). If such interactions occur in the α_1-adrenoceptor family, it might provide additional understanding of the biological importance of the C-terminal splice variants which have been reported (Hirasawa et al., 1995; Chang et al., 1998).

14. Future directions

Despite the enormous progress already made in identification and analysis of α_1-adrenoceptor subtypes, there are still many important questions to be answered. Development of more selective agonists and antagonists will allow a clearer understanding of the tissue distribution and functional roles of individual subtypes. It will be important to determine whether multiple subtypes coexist on single cells, and the impact this might have on signals generated by individual subtypes. The mechanisms by which these receptors activate mitogenic responses, and their relationship to known signaling pathways is an area of intense investigation. The possibility of novel subtype-specific signaling pathways, possibly involving direct interactions of these receptors with additional signaling proteins such as Homer, is very exciting. Potential regulation of such interactions by agonists and/or by other proteins or signaling molecules raises the possibility of completely new mechanisms regulating signaling by these receptors. The possible role of C-terminal splice variants of α_{1A}-adrenoceptors in controlling such interactions, and the possible existence of analagous splice variants for α_{1B} and α_{1D} subtypes remains to be determined. Despite continuing progress, there is much yet to be discovered.

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