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Cardiac α_1-Adrenoceptors: An Overview*

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I. Introduction

The sympathetic nervous system is a major regulator of myocardial function (for review, see Levy and Martin, 1989). For many years, β-adrenoceptors had been considered as the exclusive adrenergic receptor population through which catecholamines exert their actions on cardiac muscle. However, during the last two decades, α_1-adrenoceptors have also been identified in myocardial tissue. Selective stimulation of animal and human α_1-adrenoceptors modulates various steps of the cardiac excitation-contraction coupling cascade including ionic conductances, cytosolic ionic activities, cellular metabolism, and the Ca^{2+} sensitivity of contractile proteins. By affecting different electromechanical processes, under physiological or pathophysiological conditions, α_1-adrenoceptors could regulate the cardiac rhythm, conduction, and force of contraction. In addition to these acute actions, α_1-adrenoceptors also mediate several long-lived

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effects which include the expression of genes responsible for cell growth.

The purpose of recent investigations has been to uncover the mechanisms that underlie the α_1-adrenoceptor-mediated regulation of cellular processes in the heart. Although it has been described that α_1-adrenergic stimulation modulates cardiac contractility in a way that differs from conventional cardiotonic agents (Pucéat et al., 1992), many questions regarding the α_1-adrenergic regulation of heart function remain unresolved.

The following overview provides an update relative to the α_1-adrenoceptor-mediated effects on cardiac tissue. It is not intended to be exhaustive, and the interested reader is referred to earlier presentations of this subject for more detailed information (Brückner et al., 1985; Osnes et al., 1985; Benfey, 1987; Nawrath, 1989; Endoh, 1991; Rosen et al., 1991).

II. Characterization of Myocardial α_1-Adrenoceptors

A. Demonstration, Species Differences, and Developmental Changes of Cardiac α_1-Adrenoceptors

The unequivocal identification of myocardial α_1-adrenoceptors was made during the last decade, when investigators were able to label these receptors with specific radioligands and subsequently to isolate and clone the receptor molecule. In the late 1970s, it was demonstrated that a tritiated α-adrenoceptor antagonist ([^{3}H]dihydroergocryptine) binds specifically and with high affinity to a membrane fraction derived from myocardial multicellular preparations (rat heart: Williams and Lefkowitz, 1978; Guicheney et al., 1978; rabbit heart: Schümann and Brodde, 1979). The tritiated ligand could be displaced from specific membrane-binding sites by unlabeled α-adrenoceptor agonists and antagonists. More recently, α-adrenoceptors also have been demonstrated in isolated cardiomyocytes, a pure myocardial preparation free of any vascular or neuronal elements (Buxton and Brunton, 1986).

It was demonstrated, using α_1-subtype-selective radioligands (e.g., [^{3}H]prazosin, [^{125}I]IBE 2254), that cardiac α-adrenergic binding sites belong to the α_1-type (Steinberg and Bilezikian, 1982; Mukherjee et al., 1983). With the aid of the photoaffinity ligand [^{125}I]arylazidoprazosin, Terman and Insel (1986) identified the α_1-adrenoceptor of rat cardiomyocytes as a 77-kDa protein.

The density of α_1-adrenoceptors varies with species. Rat and rabbit myocardia possess a high density of α_1-adrenoceptor-binding sites when compared with other species. The density of binding sites in sarcolemma-enriched membrane fractions of rat, rabbit, dog, and feline hearts is 167, 191, 55, and 15 fmol/mg proteins, respectively (Mukherjee et al., 1983). Buxton and Brunton (1986), using [^{3}H]prazosin as a ligand, estimated that an adult rat ventricular cardiac cell possesses 8×10^4 α_1-adrenoceptors, or 13 α_1-receptors/μm^2. This number of α_1-adrenoceptors is comparable to the density of β_1-adrenoceptors (33/μm^2) on rat cardiomyocytes (Buxton and Brunton, 1985b). In addition, Endoh et al. (1991) showed that the ratio of α_1- to β-receptors was on average 5-fold larger in the rat than in the rabbit or dog. Although the number of α_1-adrenoceptors varies between species, no significant difference in the density of [^{3}H]prazosin-binding sites was found between the left ventricular subepicardium or subendocardium and the right ventricle of the rat heart (Muntz et al., 1985). However, in several species ventricular tissue possesses a higher density of α_1-adrenoceptors than does the atrium (Steinfath et al., 1992a).

Developmental changes in the density of myocardial α_1-adrenoceptors were observed in rabbit, rat, and dog hearts. In all three species studied, α_1-receptor density in the newborn was greater than that found in the adult. For example, in canine hearts, α_1-adrenoceptors are 10-fold more abundant in the young than in the adult heart. Specifically, using [^{125}I]IBE2254 as a ligand, del Balzo et al. (1990) found 220 fmol/mg α_1-adrenoceptors in 1-month-old dogs versus 23 fmol/mg in the adult. Using the same ligand, Buchthal et al. (1987) reported the presence of high- and low-affinity sites. The density of high-affinity sites displays no change with age (B_{max} 23 ± 6 fmol/mg in fetal, 14 ± 10 fmol/mg in neonatal, and 25 ± 15 fmol/mg in adult), whereas the density of low-affinity sites decreases in the adult (B_{max} 1460 ± 380 fmol/mg in fetal, 1710 ± 440 fmol/mg in neonatal, and 510 ± 155 fmol/mg in adult). No age-related differences in the receptor affinity have been described (Buchthal et al., 1987; Nakanishi et al., 1989; Han et al., 1989; del Balzo et al., 1990). Beyond middle age, the number of α_1-adrenoceptors further declines (Kimball et al., 1991). This decline may be due to diminished levels of α_1-adrenoceptor gene transcripts in the aging myocardium because levels of α_1-adrenoceptor mRNA as determined by Northern blot analysis decrease with age (Kimball et al., 1991).

B. Cardiac α_1-Adrenoceptor Subtypes

Evidence has been obtained from several tissues that α_1-adrenoceptors can be further subdivided into at least two pharmacologically distinct subtypes that appear to be linked to different signal transduction pathways and effector systems (Han et al., 1987; Minneman, 1988). These subtypes, named α_{1A} and α_{1B}, can be distinguished on the basis of their sensitivity toward selective antagonists. The α_{1A}-subtype has a higher affinity than the α_{1B}-subtype for the antagonists 5-methyl-urapidil, WB-4101,‡ and (+)-niguldipine or the novel prazosin deriv-

‡ Abbreviations: WB-4101, 2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane; PI, phosphatidyl inositol; SZL-49, 4-amino-6,7-dimethoxy-2-quinazolinyl-4-(2-bicyclo[2,2,2]octa-2,5 dienylcarbonyl-2-piperazine; CEC, chlorethylclonidine; AMP, adenosine monophosphate; cAMP, cyclic AMP; GTP, guanosine triphosphate; IP_3, inositol trisphosphate; IP_2, inositol biphosphate; IP_1, inositol monophosphate; IP_4, inositol tetraphosphate; IP_6, inositol hexaphosphate; PIP_2, phosphoinositide diphosphate; DAG, 1,2-diacylglycerol; PKC, protein kinase C; I_{Ca}, inward Ca^{2+} current ; I_{to}, transient outward K^+ current; I_k, delayed outward K^+ current; $I_{k\ Ach}$, muscarinic activated K^+ current; pH_i, intracellular pH; ANP, atrial natriuretic peptide; pCa, − log [Ca^{2+}; MLC, myosin light chain.

ative SZL-49. The α_{1B}-subtype is irreversibly alkylated by CEC.

Based on their respective sensitivity toward selective α_{1A}-antagonists, it was concluded that 20% of α_1-adrenoceptors belong to the α_{1A}-subtype in the rat myocardium, and the remaining 80% of the binding sites could correspond to the α_{1B}-subtype (Groß and Hanft, 1988; Groß et al., 1988a). In the membrane fraction derived from rabbit ventricles, pretreatment with 10 μM CEC decreased the B_{max} of α_1-adrenoceptors, assessed by [^{3}H] prazosin, to 37% of control, suggesting that 63% of α_1-adrenoceptors in the rabbit ventricular myocardium belong to the CEC-sensitive α_{1B}-subtype (Takanashi et al., 1991). The canine myocardium also contains a subset of α_1-adrenoceptors that are sensitive to CEC. The developmental change in the density of total α_1-adrenoceptors is not associated with a change in the proportion of α_1-receptors that are sensitive to CEC (del Balzo et al., 1990).

Molecular cloning confirmed the existence of at least two subtypes of cardiac α_1-adrenoceptors encoded by two different genes (Lomasney et al., 1991b; for review, see Lomasney et al., 1991a). The deduced amino acid sequence presumes a receptor with a seven-membrane-spanning domain topography. However, the complete classification of α_1-adrenoceptors is still not fully established. Indeed, in heart tissue, Han and Minneman (1991) recently reported the persistence of low-affinity sites for niguldipine after CEC pretreatment. Thus, the existence of additional receptor subtypes in cardiac muscle is plausible. This receptor does not belong to the α_{1C}-subtype that has a high affinity for α_{1A}-selective antagonists but is partially inhibited by CEC (Cotecchia et al., 1988; Schwinn et al., 1990, 1991). A new subtype, named α_{1D}, was recently cloned using solution phase library screening (Perez et al., 1991). It should be pointed out that the relationship between cloned α_1-adrenoceptors and pharmacologically distinct subtypes is not yet completely understood.

III. Cardiac α_1-Adrenoceptor Signal Transduction Pathways

A. Coupling of Cardiac α_1-Adrenoceptors to G-Regulatory Proteins

Guanine nucleotide regulatory proteins, G-proteins, transmit the signal from seven transmembrane domain receptors to intracellular effectors (for review, see Stryer and Bourne, 1986; Gilman, 1987; Birnbaumer et al., 1990; Taylor, 1990). G-proteins cycle between an inactive guanosine diphosphate state and an active GTP state. The hormone-receptor complex catalyzes the activation of a G-protein by accelerating the release of guanosine diphosphate and the subsequent entry of GTP. A high degree of amplification can be achieved because a single hormone-receptor complex can catalyze the activation of many G-proteins.

The property, that GTP diminishes the binding of a hormone to its receptor if a G-protein is coupled to the receptor, was exploited as a strategy to define whether a G-protein is linked to the cardiac α_1-adrenoceptor. The addition of GTP, or its analogue Gpp(NH)p, causes a rightward shift and a steepening of the agonist competition curve, which reflects the competition of α_1-adrenoceptor agonists for labeled α_1-adrenoceptor-binding sites. Because the addition of GTP reduces the agonist-binding affinity, this indicates that cardiac α_1-adrenoceptors are coupled to a GTP-binding protein (Colucci et al., 1984; Buxton and Brunton, 1986; Groß et al., 1988b; Han et al., 1989; cf. Stiles et al., 1983).

Pertussis toxin interrupts hormonal signaling by ADP-ribosylating some G-proteins (e.g., G_i and G_o classes). Pertussis toxin treatment has been reported to prevent several α_1-adrenoceptor-mediated effects including the activation of the Na^+/K^+ pump (Steinberg et al., 1985; Shah et al., 1988; Rosen et al., 1989) or the positive inotropic effect (Böhm et al., 1987). However, pertussis toxin does not prevent all α_1-adrenoceptor-mediated effects. Indeed, a pertussis toxin-insensitive G-protein which could hypothetically be the 74-kDa protein identified as G_h (Im and Graham, 1990; Im et al., 1990) has been implicated in linking the cardiac α_1-adrenoceptor to phospholipase C and to the stimulation of phosphatidyl inositol turnover, at least in rat cardiac tissue (Schmitz et al., 1987c; Steinberg et al., 1989). G_h appears to be different, by its molecular mass and chromatographic behavior, from the other pertussis toxin-insensitive G-proteins, including the G_q family, usually described as regulating the isozyme β_1 of phospholipase C (Berstein et al., 1992; Blank et al., 1991; Martin et al., 1991; Im and Graham, 1990; Im et al., 1990). Thus, several G-proteins, both pertussis toxin sensitive and insensitive, presumably couple α_1-adrenoceptors to their intracellular effectors (table 1).

B. Second Messengers

It is now well established that various molecules could serve as second messengers to convey the signal from the activated receptor-G-protein complex to different intracellular targets. These include cAMP, cylic guanosine monophosphate, and IP_3 (Sutherland, 1972; Berridge and Irvine, 1989).

In heart muscle, α_1-adrenoceptor agonists were reported not to affect either basal cAMP or cyclic guanosine monophosphate levels (Osnes and Øye, 1975; Brodde et al., 1978; review: Osnes et al., 1985; cf. Keely et al., 1977). α_1-Adrenoceptor agonists caused a decrease in cAMP levels but only under conditions in which cAMP was elevated by the prior application of a β-adrenoceptor agonist (Watanabe et al., 1977; Buxton and Brunton, 1985a). This effect was attributed to α_1-adrenergic stimulation of the cAMP-phosphodiesterase activity, because it did not take place in the presence of phosphodiesterase

TABLE 1
Pertussis toxin-sensitive and -insensitive α_1-adrenergic effects reported in cardiac tissue

Pertussis toxin sensitive	Pertussis toxin insensitive	References
Negative chronotropy		Steinberg et al. (1985)
Na/K pump activation		Shah et al. (1988)
	Positive chronotopy	Han et al. (1989)
Modulation of intracellular Ca and cell shortening		Sen et al. (1990)
	PLC	Steinberg et al. (1989)
	PI turnover	Schmitz et al. (1987c)
	I_{to}, I_{k1}, I_{kACh}	Braun et al. (1990, 1992)
		Fedida et al. (1991)
		Lee et al. (1991)
	Positive inotropic effect	Böhm et al. (1987)
Positive inotropic effect		Kim et al. (1987)
	Induction of the *Egr1* gene	Iwaki et al. (1990)

inhibitors. In rat ventricular cardiac myocytes, it was recently demonstrated that CEC completely inhibits the α_1-adrenergic effect on cAMP, suggesting that the occupation of α_{1B}-receptors leads to the activation of cAMP breakdown (Hilal-Dandan et al., 1991).

The first evidence that the adrenergic system regulates phosphoinositide metabolism (PI) in the heart came from the work of Gaut and Huggins (1966). After radiolabeled Na^+ orthophosphate was administered in vivo, epinephrine increased the radioactivity of the PI fraction of cardiac phospholipids. In 1985, Brown et al. showed that the addition of norepinephrine to [^{3}H]inositol-labeled rat ventricular cardiomyocytes caused a rapid (significant at 5 min) and prolonged (at least 40 min) increase in [^{3}H]inositol phosphate formation. The stimulatory effect of norepinephrine was maximal (5-fold the control level of [^{3}H]inositol phosphate) at 30 μM, with an EC_{50} of 1 μM. The α_1-adrenoceptor antagonist, prazosin, antagonized this effect.

Subsequently, it was confirmed that α_1-adrenergic agonists stimulate PI breakdown in different cardiac preparations. These include embryonic chick heart cells (Brown and Jones, 1986), rat perfused heart (Woodcock et al., 1987), rat ventricles (Poggioli et al., 1986), cultured rat myocardial cells (Steinberg et al., 1987), rat papillary muscles (Otani et al., 1988), rat atria (Scholz et al., 1988; Kohl et al., 1990), and canine cardiomyocytes (Heathers et al., 1989). The α_1-adrenoceptor-mediated PI turnover is not affected by the composition of the membrane's phospholipids in polyunsaturated fatty acids (Meij et al., 1990). Endoh et al. (1991) reported a correlation between the acceleration of PI turnover and the density of sarcolemmal α_1-adrenoceptors. This could explain the variations in the magnitude of the α_1-adrenergic effect on PI breakdown between species.

Poggioli et al. (1986) showed that α_1-adrenoceptor stimulation of rat muscles resulted in a significant breakdown of PIP_2 that was concomitant with a maximum increase in IP_3 formation within 30 s. Otani et al. (1988) further extended these results. The addition of neomycin, a PIP_2 phospholipase C inhibitor, inhibited the phenylephrine-induced formation of [^{3}H]inositol phosphates. 2,3-Diphosphoglyceric acid is a competitive inhibitor of IP_3 phosphatase (the enzyme that hydrolyzes IP_3 into IP_2). The combined addition of 2,3-diphosphoglyceric acid with phenylephrine doubled the IP_3 formation. In these experiments, the IP_3 fraction was not separated further.

To resolve what individual inositol phosphate isomers are formed following α_1-adrenoceptor occupation, Steinberg et al. (1989) used high-performance liquid chromatography and demonstrated that norepinephrine, in cultured rat ventricular myocytes, produced a rapid, transient increase in 1,4,5-IP_3 which was followed by a slower, sustained increase in 1,3,4-IP_3, IP_2, and IP_1. 4-IP_1 was the predominant IP_1 isomer formed during stimulation with norepinephrine. IP_2 and IP_3 accumulation was greater than IP_1 accumulation in response to α_1-adrenoceptor stimulation. This suggests that phosphoinositides, rather than PI, are the prime targets of norepinephrine-stimulated phospholipase activity in the heart.

To quantify inositol phosphate fractions in canine isolated cardiomyocytes stimulated with norepinephrine, Heathers et al. (1988) used gas chromatography coupled with high-performance liquid chromatography and showed that α_1-adrenoceptor agonists increase, within 30 s, 1,4,5-IP_3 from a baseline level of 10 to up to 40 pmol/mg protein and IP_4 from 3 to 15 pmol/mg protein. More recently, using a radioimmunoassay, Mouton et al. (1991) reported that α_1-adrenergic stimulation of isolated perfused heart increases 1,4,5-IP_3 from a basal value of 674 ± 75 to 2387 ± 385 pmol/g dry heart weight within 30 s.

Kohl et al. (1990), using electrically driven rat left atria labeled with [^{3}H]inositol, studied in more detail the time course of the effects of phenylephrine on individual inositol phosphate isomers. 1,4,5-IP_3 was the first compound to increase maximally within 30 s and to remain elevated for at least 5 min; this increase was followed by an increase in inositol tetrakisphosphate, 1,3,4,5-IP_4, and 1,4-IP_2 beginning within 2 min. The increase in 1,3,4-IP_3 and 1-IP_1 was slower and did not reach steady state

within 15 min. Thus, in addition to 1,4,5-IP_3, α_1-adrenoceptor stimulation elevates 1,3,4,5-IP_4 in rat atria. Guse et al. (1989) also showed that 1,4,5-IP_3 reached a peak within 30 s; this isomer remained high for several minutes, and 1,3,4-IP_3 and 1,3,4,5-IP_4 increased more slowly and then rapidly decreased toward their basal level within 5 min. Recently, Woodcock et al. (1992) reported that α_1-adrenergic stimulation induces an increase in IP_4 in cultured neonatal cells but not in intact neonatal hearts. The authors concluded that the metabolism of IP_3 occurred mainly by dephosphorylation in the intact heart despite the presence of an IP_3 kinase activity in this cardiac preparation (Renard and Poggioli, 1987); in isolated cultured neonatal cells, both dephosphorylation and phosphorylation pathways operate. These authors advise caution in interpreting data concerning the phosphoinositide turnover obtained in cultured neonatal cells, because these cells could have lost, in part, their cellular differentiation. The physiological role of 1,3,4,5-IP_4 in the heart remains to be determined.

Using the high-performance liquid chromatography metal dye detection technique, Scholz et al. (1992b) recently showed that α_1-adrenergic stimulation enhances not only 1,4,5-IP_3 and 1,3,4,5-IP_4 but also the cellular content of 1,3,4,6-IP_4 (by 2-fold) and IP_6 (by 1.5-fold) in isolated perfused hearts. These effects were concentration dependent, reaching a maximum at 100 μM phenylephrine for IP_3 and IP_4 and at 10 nM for IP_6. IP_3 increased at 1 min, 1,3,4,5-IP_4 increased at 2 min, and 1,3,4,6-IP_4 and IP_6 were significantly augmented at 5 min.

We would like to draw attention to the fact that, in most studies related to the α_1-adrenoceptor-mediated increase in labeled inositol phosphate, LiCl (10 mM) was used to inhibit inositol phosphatases. Even if this experimental approach is very useful in such experiments, it should be kept in mind that its use may not accurately reflect the turnover of PI as it occurs in vivo.

Some pathological conditions enhance the effect of α_1-adrenoceptor agonists on PI metabolism. For example, Xiang and McNeil (1991) observed a higher formation of IP_3 in response to α_1-adrenoceptors in diabetic than in control rats. A greater increase, by α_1-adrenoceptor stimulation, of 1,4,5-IP_3 was reported in ventricular trabeculae isolated from malignant hyperthermia-susceptible swine when compared with healthy ones (Scholz et al., 1991). Hypoxia also affects the α_1-adrenergic effect on PI turnover. Canine myocytes exposed for 10 min to hypoxia exhibit an increase in the production of IP_3 in response to submaximal concentrations of norepinephrine; the EC_{50} for norepinephrine stimulation in hypoxic cells was found to be 6-fold lower than in normoxic cells (Heathers et al., 1989). In neonatal rat ventricular myocytes, Kagiya et al. (1991b) observed an increase in α_1-adrenoceptor-induced inositol phosphate formation during the first hour of hypoxia. This effect was abolished by a prolonged hypoxia, whereas the basal level of inositol phosphates increased. In contrast, using the same cellular model, Steinberg and Alter (1993) observed a persistent enhancement of α_1-adrenoceptor-mediated increase in inositol phosphate by hypoxia up to 6 h. This effect was attributed to the stimulation of the α_{1A}-receptor subtype.

Regarding the other limb of the PI pathway, Okumura et al. (1988) directly measured the formation of DAG in response to the application of α_1-adrenoceptor agonists. α_1-Adrenoceptor stimulation produced an increase in DAG accumulation in the myocardium. DAG was measured in vivo in rat hearts using thin-layer chromatography and a flame ionization technique. Bordoni et al. (1991) also demonstrated a DAG accumulation induced by α_1-adrenoceptor agonists in cultured neonatal cardiomyocytes.

α_1-Adrenergic stimulation increases PKC activity and induces the translocation of this kinase from the cytosol to the sarcolemma (Henrich and Simpson, 1988; Mochly-Rosen et al., 1990; Kaku et al., 1991; Otani et al., 1992; Talosi and Kranias, 1992). In addition, Mochly-Rosen et al. (1990), using an immunofluorescence technique, reported that specific isozymes of the kinase were translocated to specific sites inside the cell (membrane, myofilaments, and nucleus). α_1-Adrenergic agonists induced the translocation of the Ca^{2+}-insensitive PKC isoform ϵ to the sarcolemma in both neonatal and adult cardiomyocytes (Bogoyevitch et al., 1993; Pucéat et al., 1993b).

The hydrolysis of phosphatidylcholine is currently emerging as a novel transduction pathway activated by hormones that accelerate PI turnover (Slivka et al., 1988; for reviews, see Billah and Anthes, 1990; Exton, 1990). Phosphatidylcholine breakdown is catalyzed by phospholipase A_2, phospholipase C, and phospholipase D.

In numerous tissues, α_1-adrenergic stimulation has been reported to activate phospholipases A_2 (Slivka and Insel, 1987; Weiss and Insel, 1991; for reviews, see Axelrod et al., 1988 and Insel et al., 1991). The hydrolysis of phosphatidylcholine by phospholipase A_2 releases arachidonic acid. Arachidonic acid can also be generated by the degradation of DAG following phospholipase C-induced PIP_2 hydrolysis. Arachidonic acid can activate PKC by a mechanism different from DAG (for review, see Bell and Burns, 1991); more specifically, this activation does not require phospholipids. Therefore, it has been speculated that arachidonic acid, as well as other *cis*-unsaturated fatty acids generated by the hydrolysis of phospholipids, could under physiological conditions directly activate cytosoluble PKC without inducing the kinase translocation (Khan et al., 1992). Arachidonic acid is also further metabolized inside the cell through three pathways: (*a*) the cyclooxygenase pathway leading to the formation of prostaglandins, (*b*) the epoxygenase pathway leading to the generation of epoxides, and (*c*) the lipoxygenase pathway which generates the leukotrienes. It has been reported that arachidonic acid or its

metabolites could be involved in the α_1-adrenoceptor-mediated effect seen in cardiac muscle (Molderings and Schümann, 1987; Kurachi et al., 1989, 1992).

Phospholipase C-mediated hydrolysis of phosphatidylcholine leads to a direct formation of DAG, whereas phospholipase D activation generates phosphatidic acid, which can be metabolized to DAG. Phosphatidic acid alone could also serve as a genuine second messenger because phosphatidate-dependent phosphorylations have been reported in several tissues including the heart (Bocckino et al., 1991). Data are now available to support the idea that phospholipase D can be coupled to receptors (for review, see Thompson et al., 1991). Such a hypothesis could potentially be applied to cardiac α_1-adrenoceptors. Moreover, PKC has been shown to activate phospholipase D in numerous tissues (Martinson et al., 1990; Conricode et al., 1992). These pathways could be of great physiological importance because sarcolemma contains much more phosphatidylcholine than phosphoinositides. Moreover, DAG generated through these pathways could be responsible for a sustained activation of PKC.

IV. Cellular Effects Resulting from the Stimulation of Cardiac α_1-Adrenoceptors

A. Effects on the Cardiac Action Potential and Ionic Currents

Using conventional microelectrode techniques, Pappano (1971) demonstrated that the action potential (at 90% repolarization) of guinea pig atria is prolonged by catecholamines in a propranolol-insensitive manner. Following this report, α_1-adrenergic stimulation has been repeatedly shown to increase the duration of cardiac action potentials in different multicellular myocardial preparations from various species, including sheep and dog Purkinje fibers (Giotti et al., 1973; Rosen et al., 1977), rabbit atria (Miura and Inui, 1984), rabbit papillary muscles (Handa et al., 1982), and bovine ventricular trabeculae (Brückner and Scholz, 1984). In contrast to these preparations, the guinea pig ventricle was found either to be unresponsive (Ledda et al., 1980; Hescheler et al., 1988) or to respond by a decrease in the action potential duration to α_1-adrenoceptor agonists (Dirksen and Sheu, 1990). Even in very responsive species, such as the rat, the prolongation of the action potential by α_1-adrenoceptor agonists is more pronounced in atrial than in ventricular muscle (Ertl et al., 1991).

In single, isolated ventricular myocytes, i.e., a pure cardiac preparation, catecholamines or synthetic α_1-adrenoceptor mimetics usually prolong the action potential duration (Apkon and Nerbonne, 1988; Fedida et al., 1989; Ravens et al., 1989; Vogel and Terzic, 1989). Vogel and Terzic (1989) observed a rapid increase in the action potential duration in rat cells exposed to epinephrine in the presence of propranolol and stimulated at 0.15 Hz at 37°C. This effect was concentration dependent, and 1 or 3 μM epinephrine caused the action potential duration at 90% repolarization to increase by 56%. Prazosin (100 nM) inhibited, whereas lithium chloride (10 mM) potentiated, epinephrine's action.

Little is known about the identity of the α_1-adrenoceptor subtype involved in the modulation of action potential duration in cardiac cells. Lee et al. (1991) showed that in canine Purkinje fibers the WB-4101-sensitive α_{1A}-receptor subtype mediates the prolongation of repolarization via a pertussis toxin-insensitive pathway.

In contrast to β-adrenoceptor agonists, which mostly prolong the plateau phase and do not change or even shorten the final phase of repolarization (Nathan and Beeler, 1975), α_1-adrenoceptor agonists increase the action potential to a similar extent at both 20 and 90% repolarization in bovine ventricular trabeculae without affecting the amplitude of the action potential (Brückner and Scholz, 1984). The ratio of increases in the duration of action potential at 50% to increases in the duration of action potential at 90% was measured to be 0.86 in single rat cells, indicating even a slightly smaller effect of epinephrine (1 to 3 μM) on the earlier phases of the action potential repolarization (Vogel and Terzic, 1989). In rabbit atria, Ni^+, which is known to suppress Ca^{2+} current, does not affect the prolonging effect of phenylephrine at 90% repolarization. The Na^+ channel blocker, tetrodotoxin, also does not affect the prolonging effect of phenylephrine on the duration of the action potential (Miura and Inui, 1984).

Phenylephrine also prolongs or restores Ca^{2+}-dependent (slow) action potentials in partially depolarized preparations (Miura et al., 1978; Handa et al., 1982; Brückner and Scholz, 1984). It was proposed that α_1-adrenoceptor agonists increase this current to a small extent, assuming that the maximum rate of increase of the slow action potential reflects the Ca^{2+} inward current.

When the slow I_{Ca} is directly measured, increases in I_{Ca} are rarely observed even if the action potential is prolonged by α_1-adrenoceptor stimulation. Brückner and Scholz (1984), using the sucrose-gap voltage clamp technique on bovine ventricular trabeculae, found an increase in peak I_{Ca} induced by phenylephrine as well as a slowing down in the inactivation of this current. Because it is difficult in multicellular preparations to separate the Ca^{2+} current from overlapping outward K^+ currents, it may also be difficult to determine whether a net increase in inward current resulted from an increase in inward current or from a decrease in outward currents. Apkon and Nerbonne (1988), Hartmann et al. (1988), Hescheler et al. (1988), Ravens et al. (1989), Ertl et al. (1991), Boutjdir et al. (1992), Fedida and Bouchard (1992), Jahnel et al. (1992b), and Terzic et al. (1992a), using the whole-cell patch clamp method in rabbit, guinea pig, feline, or rat ventricular or atrial cells, found no increase in I_{Ca} following α_1-adrenoceptor stimulation. These experiments were conducted under conditions in which overlapping outward currents were eliminated. In frog

ventricular cells, Alvarez et al. (1987) observed an increase in I_{Ca} following phenylephrine stimulation. This effect was more pronounced on the T-type Ca^{2+} current in frog atrial cells in which a 117% increase was reported compared to 48% for the L-type current (Alvarez and Vassort, 1992). Similarly, an increase in T-type Ca^{2+} current is observed in canine ventricular and Purkinje cells (Tseng and Boyden, 1989). The mechanism of action of the α_1-adrenoceptor agonist on T-type Ca^{2+} current is unknown. Recent observations of canine Purkinje cells suggest that the T-type Ca^{2+} current can be transiently increased by increasing the intracellular Ca^{2+} concentration (Tseng and Byden, 1991) as has been described in smooth muscle of the rat portal vein (Pacaud et al., 1987). More recently, phenylephrine was shown to increase the L-type Ca^{2+} current in neonatal rat ventricular cells, an effect that occurred within 20 min (Liu et al., 1992).

In rat ventricular myocytes, α_1-adrenoceptor agonists decrease the L-type I_{Ca} when this Ca^{2+} current is enhanced by β-adrenoceptor stimulation or by forskolin (Boutjdir et al., 1992). However, α_1-adrenoceptor agonists do not inhibit I_{Ca} if this current is increased by intracellular perfusion of cAMP, even though these agonists may stimulate the cAMP-phosphodiesterase (Buxton and Brunton, 1985a). Boutjdir et al. (1992) proposed that the α_1-adrenoceptor-mediated inhibition of β-adrenoceptor-activated I_{Ca} is due to an inhibitory G-protein coupled to adenylate cyclase.

α_1-Adrenergic agonists decrease K^+ outward currents in cardiomyocytes isolated from rat (Apkon and Nerbonne, 1988; Ravens et al., 1989; Tohse et al., 1990; Ertl et al., 1991; Fedida and Bouchard, 1992) and rabbit hearts (Fedida et al., 1989, 1990). Specifically, it has been reported that α_1-adrenergic stimulation decreases both the peak and the late current component of the (time-dependent) I_{to} (Wang et al., 1991). Fedida et al. (1989) suggested that the decrease in I_{to} could provide an explanation for the α_1-adrenoceptor-induced increase in the action potential duration. Inositol phosphates, PKC, and a pertussis toxin sensitive G-protein appeared not to be involved in transducing the α_1-adrenoceptor-mediated inhibition of I_{to} (Braun et al., 1990; Tohse et al., 1990). Stimulation of both α_1-adrenoceptor subtypes, α_{1A} and α_{1B}, contributes to the phenylephrine-induced reduction in I_{to} of isolated rat myocytes (Wang et al., 1991). Specifically, stimulation of both adrenoceptor subtypes is required for the reduction of the peak current component of I_{to}, whereas stimulation of either the α_{1A}- or the α_{1B}-subtype is sufficient for the reduction of the late current component (Wang et al., 1991).

Following 4-aminopyridine treatment to block I_{to}, α_1-adrenoceptor agonists decrease the magnitude of two inward rectifying K^+ currents: (*a*) the inwardly rectifying background current, I_{k1}, and (*b*) the muscarinic-activated, $I_{k\,Ach}$ (Fedida et al., 1991; Braun et al., 1992). The α_1-adrenergic effect on inward rectifying K^+ channels (I_{k1} and $I_{k\,Ach}$) was reported to be insensitive to pertussis toxin and does not involve the activation of PKC (Fedida et al., 1991; Braun et al., 1992). α_1-Adrenergic agonists also reduce the steady state current and I_k in rat cardiomyocytes (Ravens et al., 1989; Tohse et al., 1990; Jahnel et al., 1991) and decrease the background K^+ conductance in Purkinje fibers (Shah et al., 1988).

In guinea pig, contrary to rat, ventricular myocytes, phenylephrine (10 to 30 μM) increased the I_k (Tohse et al., 1987b, 1992). This effect was observed when intracellular Ca^{2+} was clamped to pCa 8. It was reproduced by PKC activators, occluded by pretreatment with maximally effective concentrations of PKC activators, and blocked by PKC inhibitors (Tohse et al., 1987b, 1992). Hence, this α_1-adrenoceptor-mediated increase in I_k may be related to an activation of PKC. The increase in I_k could explain why α_1-adrenoceptor agonists decrease the action potential duration in guinea pig ventricular myocytes (Dirksen and Sheu, 1990). The difference between guinea pig ventricle and other species with respect to the α_1-adrenergic effects on the duration of action potentials may be due to the absence of I_{to} channels (presumed to be responsible for the prolonging effect observed in the rat, rabbit, and other species) from guinea pig ventricular cells (Tohse et al., 1992).

In guinea pig atria, Kurachi et al. (1989) demonstrated that α_1-adrenoceptor stimulation activates the $I_{k\,Ach}$. Phenylephrine-induced activation was prevented by nordihydroguaiaretic acid, a lipoxygenase inhibitor, and AA-861, a 5-lipoxygenase inhibitor, but was not affected by indomethacin, a cycloxygenase inhibitor. It was concluded that 5-lipoxygenase metabolites of arachidonic acid may be involved in the α_1-adrenergic activation of $I_{k\,Ach}$ (reviewed by Kurachi et al., 1992).

In addition to K^+ currents, a recent study performed in guinea pig ventricular cells suggested that the α_1-adrenoceptor agonist norepinephrine, in the presence of the β-blocker propranolol, activated a chloride conductance (Walsh, 1991; Ackerman and Clapham, 1993). This chloride conductance was PKC dependent.

In summary, α_1-adrenoceptor agonists modulate several conductances in heart muscle. In some cases, this regulation does not depend on PKC or a pertussis toxin-sensitive G-protein; in others, it does, suggesting a multiplicity of subcellular coupling processes (for review, see Endoh, 1991).

The effects of the α_1-adrenoceptor stimulation on the resting membrane potential varies with the tissue. Indeed, it has been reported that α_1-adrenoceptor stimulation depolarizes, hyperpolarizes, or does not change the resting membrane potential. Miura and Inui (1984) showed that α_1-adrenoceptor stimulation produces a partial depolarization of the resting membrane potential in the rabbit atrium. More recently, Jahnel et al. (1991) also observed an α_1-adrenoceptor-mediated sarcolemmal

depolarization in rat heart atria. This depolarization was attributed to the decrease in K^+ currents in the presence of a depolarizing Na^+ inward current. However, in multicellular ventricular preparations or in Purkinje myocytes, α_1-adrenoceptor stimulation hyperpolarizes the membrane (Tohse et al., 1987b; Shah et al., 1988). Whereas Tohse et al. (1987b) reported that the Na^+/K^+ pump was not involved in this α_1-adrenoceptor mediated effect, Shah et al. (1988) and Ertl et al. (1991) attributed this hyperpolarizing action to the stimulation of the Na^+/K^+ pump because, in their experimental conditions, it was abolished by digitalis glycosides. A ouabain-sensitive hyperpolarization induced by α_1-adrenoceptor stimulation has also been reported in rat atrial muscle (Terzic et al., 1991). In isolated rat ventricular myocytes, no significant effect on resting membrane potential has been observed following the addition of α_1-adrenoceptor agonists (Ertl et al., 1991). Ertl et al. (1991) suggested that cells in isolation respond differently to a Na^+/K^+ pump stimulation than do cells in their natural environment.

B. Effects on Intracellular H^+, Na^+, and Ca^{2+} and on Ionic Transport Mechanisms

α_1-Adrenoceptor agonists produce an intracellular alkalinization. This finding has been described in atria (Terzic et al., 1991), perfused hearts (Fuller et al., 1991), single isolated ventricular cardiomyocytes (Astarie et al., 1991; Gambassi et al., 1992; Terzic et al., 1992a; Pucéat et al., 1993a), cardiac cells in suspension (Iwakura et al., 1990; Wallert and Fröhlich, 1992), and Purkinje fibers (Breen and Pressler, 1988; Pressler et al., 1989; see, however, Guo et al., 1992). To measure pH_i, ion-selective microelectrodes (Terzic et al., 1991) and pH_i-sensitive fluorescent indicators (Iwakura et al., 1990; Gambassi et al., 1992; Terzic et al., 1992a; Wallert and Fröhlich, 1992) have been used. In addition, [^{14}C]5,5′-dimethyloxazolidine-2,4-dione, a compound that partitions between the intracellular and extracellular spaces as a function of intracellular and extracellular pH, was used to assess pH_i (Fuller et al., 1991).

Both synthetic sympathomimetics and endogenous catecholamines (in the presence of β-adrenoceptor blockers) induce an alkalinization that typically amounts to 0.1 pH units at 30 μM epinephrine or 100 μM phenylephrine either in bicarbonate-poor or -rich bathing solutions (Astarie et al., 1991; Fuller et al., 1991; Terzic et al., 1992a). The selective α_1-adrenoceptor blocker, prazosin, but not the α_2-adrenoceptor blocker, yohimbine, abolished this alkalinization, indicating that the sarcolemmal α_1-adrenoceptor is responsible for the effect on pH_i (Terzic et al., 1992a; Wallert and Fröhlich, 1992).

The origin of the alkalinization has been ascribed to the stimulation of Na^+/H^+ exchange, a major alkalinizing transporter. Three findings support that conclusion: (*a*) selective inhibitors of Na^+/H^+ exchange (e.g., ethylisopropylamiloride, hexamethylamiloride) abolish or prevent the α_1-adrenoceptor-mediated alkalinization (Iwakura et al., 1990; Terzic et al., 1991; Gambassi et al., 1992; Terzic et al., 1992a), (*b*) replacement of extracellular Na^+ with *N*-methylglucamine blocks the α_1-adrenoceptor agonist-induced alkalinization (Wallert and Fröhlich, 1992), and (*c*) α_1-adrenoceptor agonists enhance pH_i recovery from acidosis under conditions in which this recovery primarily depends on Na^+/H^+ exchange (Terzic et al., 1992a; Pucéat et al., 1993a). Furthermore, it appears that α_1-adrenoceptor agonists do not affect the intracellular buffering capacity. A lack of α_1-adrenoceptor-mediated effects on the apparent buffering capacity was established in both the presence and absence of Na^+/H^+ antiport inhibitors (Terzic et al., 1992a; Pucéat et al., 1993a).

The results concerning pH_i reviewed above were obtained under physiological extracellular pH. α_1-Adrenoceptor agonists also produce an alkalinization and accelerate the recovery of pH_i following an imposed acid challenge via the stimulation of Na^+/H^+ exchange under extracellular acidosis (Pucéat et al., 1993a). These effects could potentially be significant under conditions associated with extracellular acidosis, such as ischemia or hypoxia. By stimulating the Na^+/H^+ antiport, α_1-adrenoceptor agonist could modulate cardiac mechanisms that are sensitive to changes in pH_i. This includes various steps responsible for cardiac contraction and cell growth.

The molecular pathway by which α_1-adrenoceptor agonists stimulate the Na^+/H^+ antiport is still not known. As in many noncardiac tissues (Frelin et al., 1988), it has been suggested that PKC is responsible for the activation of the exchanger because phorbol esters mimic, whereas PKC inhibitors (e.g., H7, staurosporine) block, the α_1-adrenoceptor agonist-mediated alkalinization in a suspension of ventricular cells or in Purkinje fibers (Sharma and Sheu, 1987; Breen and Pressler, 1988; Iwakura et al., 1990; Wallert and Fröhlich, 1992). Likewise, in cardiomyocytes preincubated with the phorbol ester, phorbol-12-myristate-13-acetate, or with staurosporine to down-regulate or inhibit PKC, respectively, α_1-adrenoceptor agonists did not produce an alkalinization (Gambassi et al., 1992). A role for Ca^{2+}-calmodulin-dependent kinase has also been proposed because W7, an inhibitor of Ca^{2+}-calmodulin-dependent kinase, also inhibits the α_1-mediated alkalinization (Iwakura et al., 1990; Wallert and Fröhlich, 1992). However, Pucéat et al., (1993a) could not confirm these results in rat single ventricular myocytes. Indeed, in this latter study, the stimulation of Na^+/H^+ exchange by α_1-adrenoceptor agonists was not affected by the presence of an intracellular Ca^{2+} chelator, suggesting that changes in intracellular Ca^{2+} are not required for these effects. Neither staurosporine nor GF109203X, two inhibitors of PKC, was able to prevent the phenylephrine-induced alkalinization. Furthermore, the α_1-adrenoceptor-triggered acceleration of pH_i recovery from an imposed acid load was not altered by stau-

rosporine. Although the signal transduction pathway linking the α_1-adrenoceptor to the activation of the Na^+/H^+ exchange still remains a question of controversy, evidence was obtained suggesting that α_1-adrenoceptor agonists produce an increase both in the apparent affinity of the Na^+/H^+ antiport for protons and in its maximal ionic exchange activity (Pucéat et al., 1993a; also see Lagadic-Gossmann et al., 1992b; Wallert and Fröhlich, 1992).

In addition to the Na^+/H^+ antiport, cardiac cells possess a bicarbonate-dependent alkalinizing transporter (Liu et al., 1990; Dart and Vaughan-Jones, 1992; Lagadic-Gossmann et al., 1992a). Selective α_1-adrenoceptor agonists, such as phenylephrine, do enhance the recovery of pH_i from acidosis under conditions in which the Na^+/H^+ antiport is blocked (Terzic et al., 1992b). This effect is absent in bicarbonate-free solutions and, thus, suggests that α_1-adrenoceptor could activate not only Na^+/H^+ exchange but also a bicarbonate-dependent, amiloride-insensitive, alkalinizing transport mechanism at least in rat ventricular cardiomyocytes (Terzic et al., 1992b). However, in guinea pig cardiac cells, epinephrine, which stimulates both α- and β-adrenoceptors, inhibits pH_i recovery from acidosis in the presence of amiloride and bicarbonate (Lagadic-Gossmann et al., 1992b). The reason underlying the difference between these two studies is unknown but could be due to opposing effects of α- and β-adrenergic stimulation on cardiac pH_i regulation.

In addition to producing an intracellular alkalinization, the activation of the Na^+/H^+ antiport by α_1-adrenoceptor agonists could be expected to increase intracellular Na^+. However, α_1-adrenoceptor stimulation also increases Na^+/K^+ pump activity leading to a decrease in intracellular Na^+ (Zaza et al., 1990; Wilde and Kleber, 1991) and an increase in K^+ uptake (Ellingsen et al., 1987). Indeed, Terzic et al. (1991) observed an increase in intracellular Na^+ only when the α_1-adrenoceptors agonist was applied in the presence of ouabain, which inhibits Na^+/K^+ pumping. It could be hypothesized that the concomitant stimulation of Na^+/K^+ pumping, and in turn Na^+ efflux, counterbalanced the increased influx of Na^+ produced by Na^+/H^+ antiport activation. Using radiolabeled ^{22}Na, Jahnel et al. (1991) reported an increase in unidirectional Na^+ influx in resting atria stimulated with phenylephrine. This effect was attributed to a depolarization-triggered activation of the tetrodotoxin-sensitive Na^+ window current because the agonist induces a depolarization of atrial cells. It can be argued that α_1-adrenergic stimulation enhances both Na^+ influx and efflux mechanisms with a slight net effect in one or the other direction.

The effects of α_1-adrenoceptor agonists on intracellular Ca^{2+} have not been elucidated unequivocally as yet. Regarding diastolic Ca^{2+}, studies using Ca^{2+}-sensitive fluorescent indicators (Indo-1, Fura-2) and performed on isolated myocytes, maintained in suspension or attached to coverslips, showed that α_1-adrenoceptor agonists moderately increased diastolic intracellular Ca^{2+}. These results were obtained in quiescent rat cells (Iwakura et al., 1990; Eckel et al., 1991) or electrically stimulated rat atrial cells (Jahnel et al., 1992b) and hamster cardiomyocytes (Sen et al., 1990). A pertussis toxin-sensitive G-protein has been implicated to link α_1-adrenoceptors to the modulation of diastolic Ca^{2+} in hamster cardiac myocytes (Sen et al., 1990). Jahnel et al. (1991) observed a significant increase in $^{45}Ca^{2+}$ uptake in beating atria stimulated with phenylephrine. Whereas Iwakura et al. (1990) and Jahnel et al. (1991, 1992b) postulated that the increase in intracellular Ca^{2+} was due to Na^+/Ca^{2+} exchange following an increase in intracellular Na^+, Eckel et al. (1991) proposed that α_1-adrenergic agonists mobilized an intracellular Ca^{2+} pool.

With regard to systolic Ca^{2+} associated with twitch contractions, Endoh and Blinks (1988) showed a small increase in Ca^{2+} transients following the application of α_1-adrenoceptor agonists to rabbit papillary muscles microinjected with aequorin. O'Rourke (1990) and Capogrossi et al. (1991) demonstrated that the ability of α_1-adrenoceptor agonists to affect systolic Ca^{2+} depends on the external Ca^{2+} concentration (O'Rourke et al., 1992). At low external Ca^{2+} concentrations (0.5 to 1 mM $CaCl_2$), α_1-adrenoceptor agonists appear to moderately increase intracellular Ca^{2+} transients (also see Fedida and Bouchard, 1992). At 1.5 mM external $CaCl_2$, 50% of myocytes show an increase in Ca^{2+} transients following α_1-adrenoceptor stimulation, whereas the other half do not (Gambassi et al., 1992). At 2 mM external $CaCl_2$, α_1-adrenoceptor agonists no longer or inconsistently increase intracellular Ca^{2+} transients (O'Rourke, 1990; Failli et al., 1992; cf. Jahnel et al., 1992b). Failli et al. (1992) reported that, of 46 single cardiac cells exposed to 2 mM $CaCl_2$, only 12 myocytes (26%) responded to phenylephrine (10 to 100 μM). At higher external Ca^{2+} concentrations (5 mM), α_1-adrenoceptor agonists actually decreased Ca^{2+} transients (Capogrossi et al., 1991; O'Rourke et al., 1992). Using spectromicrofluorometry and adjusting the external Ca^{2+} concentrations to physiological levels (1.8 mM) for the rat, Terzic et al. (1992a) observed no change in Ca^{2+} transients in electrically driven single cells superfused with phenylephrine.

C. Metabolic Effects

Epinephrine, in the presence of propranolol, can regulate glycogen and glucose metabolism in cardiac muscle (for review, see Osnes et al., 1985). Stimulation of cardiac α_1-adrenoceptors increases glucose uptake, the activity of phosphofructokinase (a rate-limiting glycolysis enzyme), and lactate formation (Keely et al., 1977; Clark and Patten, 1984). Although stimulating glycolysis, α_1-adrenoceptor agonists inhibit the enzymatic activity of glycogen synthase (Ramachandran et al., 1983). α_1-Adrenergic agonists do not, or only modestly, increase phos-

phorylase *a* activity (Clark and Patten, 1984; Osnes et al., 1985). α_1-Adrenoceptor agonists also modulate the pentose pathway which supplies precursors for adenine nucleotide synthesis. When injected for 3 days into rats, norepinephrine and norfenefrine (in the presence of β-adrenoceptors antagonists) activate up to 8-fold (in a dose-dependent and prazosin-sensitive manner) glucose-6-phosphate dehydrogenase, the regulating enzyme of the pentose pathway (Zimmer et al., 1992). The increase in glucose-6-phosphate dehydrogenase is due to an enhancement of the enzyme's mRNA levels. Zimmer et al. (1992) suggested that the stimulation of the pentose pathway by catecholamines (β-adrenoceptor agonists also stimulate glucose-6-phosphate dehydrogenase) could provide an adaptive mechanism to balance the energetic expenditure due to the positive inotropic effect of these neurotransmitters.

Mitochondrial functions, including oxygen consumption, are affected by α_1-adrenoceptor stimulation (Osnes et al., 1985). It was reported that the rate of Ca^{2+} uptake by mitochondria, isolated from hearts perfused with an α-adrenergic agonist, was significantly increased when compared with control mitochondria (Crompton et al., 1983).

In ATP-depleted rat cardiomyocytes, phenylephrine enhances the deamination of AMP into inosine monophosphate (Hohl et al., 1989). This reaction is catalyzed by adenosine deaminase.

α_1-Adrenergic agonists were also reported to stimulate protein synthesis in both isolated myocytes and perfused hearts (Fuller et al., 1990). This effect appears to depend on intracellular alkalinization induced by α_1-adrenoceptor agonists and is associated with an increase in intracellular phosphocreatine concentration (Fuller et al., 1991). It was suggested that the effects of the α_1-adrenoceptor agonist in adult cardiac tissue is exerted at the level of translation because it was not prevented by actinomycin D (Fuller et al., 1990). Thus, it can be postulated that these effects on protein synthesis can be dissociated from the effects of α_1-adrenergic stimulation on cell growth and hypertrophy in neonatal cells which occur at the level of transcription (see section V.D).

Mammalian atrial myocytes synthesize and secrete a potent natriuretic and vasoactive polypeptide hormone, termed ANP (Currie et al., 1983). Stimulation of α_1-adrenoceptors enhances ANP secretion in adult hearts (Currie and Newman, 1986; Matsubara et al., 1987; Wong et al., 1988; Christensen et al., 1991). Using an in vivo model, Lachance and Garcia (1991) also observed a phenylephrine-induced increase in circulating ANP concentration. Furthermore, these authors showed that adrenergic stimulation potentiates the ANP secretion triggered by an increase in atrial wall tension. Sei and Glembotski (1990) reported that α_1-adrenergic stimulation also triggered ANP secretion from atrial neonatal cells. A decrease in extracellular Ca^{2+} concentration to 2 nM with ethyleneglycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid or the blockade of Ca^{2+} channels with nifedipine diminished by half the phenylephrine-induced ANP secretion. Schiebinger et al. (1992) described a Ca^{2+} influx as mandatory for α_1-adrenoceptor agonists to release ANP from rat isolated atria.

Lindemann (1986) showed that a sarcolemmal 15-kDa protein was phosphorylated following the stimulation of rat ventricles with α_1-adrenoceptor agonists. Meij et al. (1991) and Hartmann and Schrader (1992) reported that this protein was also phosphorylated following the treatment of cultured neonatal and adult cardiomyocytes with phorbol esters. It was proposed that this phosphorylation could play a role in the down-regulation of the responsiveness of cardiac tissue to α_1-adrenergic stimulation. A protein with an apparent molecular mass of 15 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis has been purified, cloned, and sequenced. It has a calculated molecular mass of 8.4 kDa and was named "phospholemman" (Palmer et al., l991). It was speculated that its phosphorylation could modulate the activity of colocalized channels, pumps, and/or antiporters by altering sarcolemmal surface charges or, as recent data indicate, that this protein could be a chloride channel by itself (Moorman et al., 1992). In contrast to the studies performed in rat ventricles or isolated cells, Edes et al. (1991) failed to observe any phosphorylation of the 15-kDa protein following α_1-adrenergic or phorbol ester treatment of beating guinea pig hearts. This was probably related to the animal species they used because Talosi and Kranias (1992) showed a phosphorylation of this sarcolemmal protein following α_1-adrenergic stimulation of rabbit hearts. In addition, a cytosolic 28-kDa protein was also found to be phosphorylated following exposure to phenylephrine.

V. Physiological and Pathophysiological Consequences of α_1-Adrenoceptor Stimulation

A. α_1-Adrenoceptors and Inotropy

In 1966, Wenzel and Su were the first to report a positive inotropic effect of an α_1-adrenoceptor agonist, phenylephrine, in rat ventricular strips. Soon thereafter, other investigators observed the positive inotropic effect of various α_1-adrenoceptor agonists in rabbit and guinea pig atria (Benfey and Varma, 1967; Govier, 1967).

Stimulation of myocardial α_1-adrenoceptors produces a positive inotropic effect in different cardiac preparations (whole hearts, papillary muscles, ventricular strips, atria, isolated cardiomyocytes) from several species (rat, rabbit, guinea pig, cat, lamb, cow, dog, monkey) (Wagner and Brodde, 1978; Shibata et al., 1980; Skomedal et al., 1983; Terzic and Vogel, 1990; Fedida and Bouchard, 1992; Gambassi et al., 1992; for review, see Brückner et al., 1985; Osnes et al., 1985; Endoh, 1986, 1991; Scholz et al., 1986; Benfey, 1987; Nawrath, 1989; Pucéat et al., 1992). Skomedal et al. (1988, 1990) demonstrated a definite

contribution of the α_1-adrenoceptor to the inotropic response of heart muscle to endogenous catecholamines in the presence of unopposed β-adrenoceptors stimulation. These investigators estimated that about 75% of the response to norepinephrine is mediated through β-adrenoceptors and 25% via α_1-adrenoceptor in rat cardiac tissue. Concomitant muscarinic receptor stimulation increases the α_1-adrenoceptor component of the overall inotropic effect of norepinephrine (Christiansen et al., 1987).

As expected, selective α_2-adrenoceptor agonists cause no positive inotropic effect (Williamson and Broadley, 1987; Housmans, 1990). The selective α_1-adrenoceptor blocker, prazosin, in nanomolar concentrations, competitively inhibited the positive inotropic action of phenylephrine (Skomedal et al., 1980). The nature of the α_1-adrenoceptor subtype(s) responsible for the positive inotropic effect is a matter of current investigation. In rabbit papillary muscle, the α_1-adrenoceptor-mediated positive inotropic action is inhibited by the selective α_{1B}-adrenoceptor-alkylating agent CEC in a concentration-dependent manner (IC_{50} = 2.4 μM) and abolished by 10 μM CEC (Takanashi et al., 1991). Endoh et al. (1992) recently reported that WB-4101, the α_{1A}-subtype selective antagonist, shifted to a small extent the concentration-response curve of the positive inotropic effect induced by phenylephrine and suggested that the α_{1A}-subtype may also mediate the inotropic effect of α_1-agonists, although to a much smaller extent than the α_{1B}-receptor. Michel et al. (1990) also implicated the α_{1B}-receptor subtype in mediating the inotropic action. In contrast, preliminary recent reports suggest that the stimulation of the α_{1A}-subtype, at least in rat tissue, is responsible for the α_1-adrenoceptor-mediated positive inotropic effects in both papillary muscle (Rokosh and Sulakhe, 1991) and isolated cells (Gambassi et al., 1991). SZL-49 and WB-4101 ihibited the norepinephrine-induced increase in inotropy; CEC failed to prevent this effect.

The positive inotropic effect resulting from the activation of α_1-adrenoceptors varies in magnitude from one species to another. Larger increases in developed force are found in the rat and rabbit than in the guinea pig and dog myocardium (Scholz et al., 1986). The differences among species could be related to the density of α_1-adrenoceptors (Mukherjee et al., 1983; Endoh et al., 1991). Nakanishi et al. (1989) compared the positive inotropic effect of α_1-adrenoceptor agonists in newborn and adult rats, rabbits, and dogs. For a given agonist concentration, the effect was greater in the adult. However, beyond middle age, an aging-associated decline in the maximum positive inotropic effect of α_1-agonists was reported (Kimball et al., 1991).

1. Characteristics of the α_1-adrenergic positive inotropic effect. In some cardiac preparations, the α_1-adrenergic response exhibits a complex (biphasic, triphasic) time course including a negative inotropic component (Govier, 1968; Skomedal et al., 1983; Osnes et al., 1985; Tohse et al., 1987a; Otani et al., 1988; Ertl et al., 1991). For example, stimulation of α_1-adrenoceptors in rat papillary muscles results in a triphasic inotropic response. An initial increase in contractile force (phase 1) appears immediately, reaching a maximum level within 30 s. The contractile force then declines below the baseline, producing a negative inotropic phase (phase 2) that reaches a maximum level at 80 to 90 s. The second increase in contractile force (phase 3) is more pronounced than that of phase 1. It reaches a maximum level at 5 to 6 min and persists for a long period (>20 min). A proportion (30%) of frog atrial trabeculae responds to α-stimulation by a transient response (Niedergerke and Page, 1981).

A detailed account of the characteristics of the steady state positive inotropic effect of α_1-adrenoceptor agonists was presented by Osnes et al. (1985) and Endoh (1986). One property of the α_1-adrenoceptor-mediated positive inotropic effect is an increase in the contraction amplitude with no change or a slight prolongation in the duration of the contraction-relaxation cycle; there is also no change or a slight increase in time to peak tension and relaxation time (Ledda et al., 1975; Endoh and Blinks, 1988; Skomedal et al., 1983; El Amrani et al., 1989). Li and Rouleau (1991) recently studied rabbit papillary muscle and reported a significant increase in time to peak tension and in relaxation time. Consequently, all phases of the cycle are proportionally increased in the presence of an α_1-adrenoceptor agonist. Also, an increase in the V_{max} of unloaded muscle shortening was observed (Li and Rouleau, 1991).

Phosphodiesterase inhibitors (e.g., theophylline) and adenylate cyclase inhibitors (i.e., muscarinic and adenosine agonists) do not affect the inotropic response of cardiac muscle to α_1-adrenoceptor stimulation (Endoh and Motomura, 1979; Endoh and Yamashita, 1980; Christiansen et al., 1987; for review, see Osnes et al., 1985; Endoh, 1991). This is expected because the positive inotropic effect of α_1-adrenoceptor agonists is unrelated to cAMP.

Some experimental conditions (e.g., pacing frequency, temperature, and Ca^{2+} concentration of the bathing solution) can affect the magnitude of the positive inotropic response to α_1-adrenoceptor agonists (reviewed by Endoh, 1986). The α_1-adrenoceptor-mediated positive inotropic effect is most prominent at a low rate of muscle stimulation (0.5 Hz) and decreases or is absent at high stimulating frequencies (Endoh and Schümann, 1975; Scholz et al., 1986). Lowering the temperature of the bathing solution from 37°C to 32°C shifts the concentration-response curve for phenylephrine to the left (Endoh et al., 1977). An important modulator of the magnitude of the positive inotropic response to α_1-adrenoceptor agonists in mammalian heart is the endocardial endothelium (Meulemans et al., 1990). The positive inotropic

effect of phenylephrine in the lower concentration range (1 to 100 nM) requires the presence of an intact endocardial endothelium. Higher concentrations of phenylephrine destroy the endocardial endothelium and shift the dose-response curve of α_1-adrenergic agonists toward higher concentrations.

Increasing the Ca^{2+} concentration of the bathing solution to 5 mM results, at least in isolated ventricular cells, in a sustained negative inotropic effect to α_1-adrenoceptor agonists (Capogrossi et al., 1991). This negative inotropic effect cannot be ascribed to Ca^{2+} overload because α_1-adrenoceptor agonists suppress spontaneous Ca^{2+} release from the sarcoplasmic reticulum of isolated cells usually observed under this experimental condition. This negative inotropic effect was ascribed to an enhanced α_1-adrenoceptor-mediated activation of PKC when intracellular Ca^{2+} is increased by high external Ca^{2+} (Capogrossi et al., 1991). However, it should be pointed out that even at higher external Ca^{2+} concentrations (>7.5 mM) a positive inotropic effect of phenylephrine, albeit small, was observed in papillary or atrial muscle (Meulemans et al., 1990; Li and Rouleau, 1991; Terzic and Vogel, 1991). It is not known what could explain this difference between isolated cardiomyocytes and intact muscle. A possible explanation could be that isolated cells have a diminished tolerance to Ca^{2+}. Molderings and Schümann (1987) also reported that, under some experimental conditions, the magnitude of the increase in inotropy induced by α_1-adrenoceptor agonists could depend on the extracellular Ca^{2+} concentration. These authors showed that inhibition of cyclooxygenase increased the α_1-adrenoceptor-mediated positive inotropic effect at low agonist concentrations when the atria were bathed in 1.2 mM Ca^{2+}. This effect was not further observed when external Ca^{2+} was elevated to 2.5 mM.

2. α_1-Adrenoceptor-mediated positive inotropic effect in pathological conditions. It has been proposed that α_1-adrenoceptors might serve as a reserve mechanism to maintain myocardial responsiveness to catecholamines under conditions in which the β-adrenoceptor is blocked, functionally antagonized, reduced in number, or uncoupled from its transduction pathway (Brückner et al., 1985; Osnes et al., 1985; Homcy et al., 1991). Furthermore, several pathological and clinical situations modify the density of α_1-adrenergic receptors in the myocardium which could be associated with an increase in the positive inotropic effect induced by α_1-adrenoceptors agonists.

For example, chronic treatment with β-adrenergic antagonists augments the number of myocardial α_1-adrenoceptors (Mügge et al., 1985). This propranolol-induced increase in the density of α_1-adrenoceptors is inhibited by cycloheximide, an inhibitor of protein synthesis, suggesting that it was due to de novo receptor synthesis (Steinkraus et al., 1989). At least in rat hearts, the increase in α_1-adrenoceptors density was not accompanied by an enhancement of the positive inotropic response to α_1-adrenergic agonists (Steinkraus et al., 1989).

In congestive heart failure, β-adrenoceptors are reduced in number, by more than half, as compared with normally functioning hearts (Bristow et al., 1982). This reduction is accompanied by a decrease in the biochemical and inotropic responsiveness of cardiac tissue to β-adrenoceptor agonists (Bristow et al., 1985). No difference in the absolute density of α_1-adrenoceptors or in the α_1-adrenoceptor-mediated effects on PIs has been observed in the failing when compared to the nonfailing heart (Bristow et al., 1988). The question remains whether endogenous catecholamines could support cardiac function in heart failure via the myocardial α_1-adrenoceptor (Schmitz et al., 1987a).

Hearts isolated from cardiomyopathic Syrian hamsters show an enhanced positive inotropic response to α_1-adrenoceptors (Böhm et al., 1986; Sen et al., 1990). Horackova et al. (1991) showed that the EC_{50} for the α_1-adrenoceptor-mediated positive inotropic effect was 50% lower in cardiomyopathic than in normal hamsters. With the progression of cardiomyopathy, β-adrenoceptors gradually disappear, whereas α_1-adrenoceptor density remains high, even when heart failure develops fully (Kagiya et al., 1991a).

An increase in the α_1-adrenoceptor density was also observed in cardiac hypoxia (Heathers et al., 1988; Kagiya et al., 1991b). An explanation for this increase is not yet forthcoming. This change in density could be explained by the incorporation in the sarcolemma of newly synthesized receptors or, as previously suggested, by an unmasking of covert receptors following the modification of membrane fluidity (Heathers et al., 1988).

In hypertensive animals, both the number of cardiac β-adrenoceptors and the positive inotropic effect of β-adrenergic agonists are reduced (Böhm et al., 1988a). The positive inotropic effect of phenylephrine appears not to differ between normotensive and hypertensive animals (Fujiwara et al., 1972). Also, the total cardiac content of α_1-adrenoceptors is similar in hypertensive and normotensive animals (Limas and Limas, 1987). However, the distribution of α_1-adrenoceptors is higher in the sarcolemma and lower in the cytosolic vesicular fraction of the myocardium obtained from hypertensive animals when compared with normotensive controls (Limas and Limas, 1987). Therefore, the α/β ratio of plasmalemmal cardiac adrenoceptors is changed in hypertensive animals, with the α_1-adrenoceptor component becoming more important in hypertension.

The effect of dietary fish oil on cardiac function and responsiveness to adrenoceptor agonists has been studies in perfused rat hearts. The inotropic response to α-agonists is reduced following a 4-week diet containing 5% menhaden oil, whereas the cardiac responsiveness to β-adrenoceptor agonists is not affected by dietary fish oil (Reibel et al., 1988).

In hypothyroidism the inotropic response to α_1-adrenergic stimulation is increased (Nakashima et al., 1971; Kunos et al., 1974; reviewed by Osnes et al., 1985). In the hypothyroid state the number of α_1-adrenoceptors has been reported to be unchanged (Williams and Lefkowitz, 1979) or even reduced (Groß and Lues, 1985). Thyroid hormones modulate isozyme transition of myosin in the mammalian ventricular myocardium (Winegrad, 1984). Hypothyroidism causes a transition to the V3 isozyme, which responds to α- but not to β-adrenergic stimulation (Endoh, 1986). In addition, the transition from the V1 to the V3 myosin isoform leads to a decrease in the maximal actomyosin ATPase activity.

Experimentally induced diabetes mellitus is also characterized by an increased inotropic responsiveness of isolated cardiac muscle or whole working hearts to α_1-adrenoceptor agonists (Downing et al., 1983; Canga and Sterin-Borda, 1986; Heijnis and van Zwieten, 1992). The dose response to α_1-adrenoceptor agonists shows both a leftward and an upward shift in diabetic animals. Binding studies reveal a reduced number of α_1-adrenoceptor-binding sites associated with no change (Tanaka et al., 1992) or an increase in their affinity constants (Wald et al., 1988). The decrease in cell surface receptor density has been suggested to be linked to a high cardiac PKC activity, also observed in diabetic models (Tanaka et al., 1992).

3. Proposed mechanisms of the α_1-adrenergic positive inotropic effect. Do α_1-adrenoceptor agonists belong to a traditional positive inotropic group of agents? They do not (Pucéat et al., 1992). They differ from β-adrenoceptor agonists, phosphodiesterase inhibitors, H_2-histamine agonists, glucagon, and other positive inotropic agents that increase contractile force by elevating cAMP levels. Unlike dihydropyridine agonists (e.g., Bay K 8644), α_1-adrenoceptor agonists do not directly activate I_{Ca}. Also, α_1-adrenoceptor agonists do not share the inotropic mechanism with cardiotonic glycosides because they do not inhibit Na^+/K^+ pumping. Several mechanisms have been proposed to participate in the positive inotropic effect of α_1-adrenoceptor agonists (fig. 1). Currently, three of these mechanisms are being investigated: (*a*) an indirect increase in I_{Ca} inward current, (*b*) a stimulation of inositol phosphate turnover, and (*c*) an increase in myofibrillar responsiveness to Ca^{2+}.

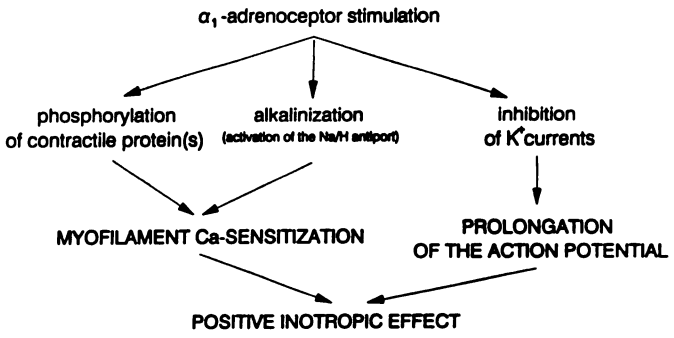

FIG. 1. Proposed mechanisms underlying the positive inotropic action of α_1-adrenoceptor agonists.

a. EVIDENCE FOR AND AGAINST A CAUSAL RELATIONSHIP BETWEEN α_1-ADRENERGIC EFFECTS ON THE ACTION POTENTIAL AND POSITIVE INOTROPIC EFFECT. Because there is a known relationship between the duration of action potentials and contractile force, it is natural to consider that the two are related when the action potential is prolonged by α_1-adrenoceptor agonists. A prolonged action potential due to the inhibition of I_{to} by α_1-adrenoceptor agonists will increase Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Fedida et al., 1989). Because of the properties of I_{to}, such a mechanism could provide an explanation for the frequency dependency of the positive inotropic effect of α_1-agonists. Indeed, preexposure of cardiac cells to 4-aminopyridine, a blocker of I_{to}, appears to prevent α_1-agonists from producing a positive inotropic effect (Tohse et al., 1990). In addition, Ca^{2+} channel antagonists (verapamil, diltiazem, and nifedipine) have been shown to block, at least to some extent, the positive inotropic effect of α_1-adrenoceptor agonists (Tohse et al., 1987a; Kushida et al., 1990; Endou et al., 1991). These findings could be explained by a dependency of the positive inotropic effect of α_1-adrenoceptor agonists on an increase in Ca^{2+} influx. However, such an explanation should be viewed with caution because Ca^{2+} channel blockers also decrease basal contractile force and exhibit an affinity for cardiac α_1-adrenoceptor-binding sites (Kushida et al., 1990).

Recently, Fedida and Bouchard (1992), using the whole cell voltage clamp technique to control the duration of depolarization, provided evidence that, at least under the experimental conditions used, the increase in contractile force produced by α_1-agonists can be observed only when the action potential duration is increased. Although the results of these experiments point out that the prolongation of the action potential plays an important role in the positive inotropic effect of α_1-adrenoceptor agonists, they do not necessarily mean that additional inotropic mechanisms do not also participate in the inotropic action of α_1-agonists. A potential limitation of the technique used could have been that the internal dialysis of myocytes through the patch pipette may have prevented the mechanisms leading to myofibrillar sensitization to Ca^{2+} to take place.

Several reports suggest that the electrophysiological effects caused by α_1-adrenoceptor agonists can be dissociated from their positive inotropic effects. For example, in normally polarized myocardial preparations exposed to Mn^{2+}, which causes a suppression of the slow inward current, phenylephrine produced a marked prolongation of the action potential duration without an increase in contractile force (Handa et al., 1982). In addition, for concentrations of α_1- and β-adrenoceptor agonists that produce the same magnitude of positive inotropic effect, α_1-adrenoceptor agonists have a much smaller effect on

the maximum rate of depolarization of slow action potentials as compared to β-adrenoceptor agonists (Brückner and Scholz, 1984). Furthermore, for a given depolarizing pulse (i.e., under voltage clamp conditions), at least in feline cardiac cells, α_1-adrenoceptor stimulation produces an increase in contractile force with no increase in Ca^{2+} current (Hartmann et al., 1988). Dirksen et al. (1991) reported that, in guinea pig ventricle, α_1-adrenoceptor stimulation produces a positive inotropic effect even when the Ca^{2+} transient and the action potential duration are decreased. Similarly, in rat cardiac cells, a positive inotropic effect of phenylephrine can be observed, at least in some cells, in the absence of an increase in intracellular Ca^{2+} (Gambassi et al., 1992). It might be argued on these grounds that the prolongation of the action potential might participate, but is not the sole origin, of the α_1-adrenoceptor-mediated increase in cardiac contractility.

b. IS THERE A CAUSAL RELATIONSHIP BETWEEN α_1-ADRENOCEPTOR-MEDIATED STIMULATION OF THE BREAKDOWN OF PHOSPHATIDYL INOSITOLS AND CONTRACTILE FORCE? As reviewed above, the stimulation of cardiac α_1-adrenoceptors promotes the breakdown of PI, producing IP_3 and DAG. The role of these molecules in the excitation-contraction process in heart muscle, as well as in mediating the positive inotropic effect of α_1-adrenoceptor agonists, is not yet fully clarified.

A prerequisite for a second-messenger role of IP_3 in the α_1-adrenoceptor-mediated inotropic action is that the α_1-adrenoceptor agonist-induced increase in IP_3 should precede the increase in the force of contraction. Indeed, Schmitz et al. (1987b) and Scholz et al. (1988) found that the positive inotropic effect of phenylephrine in rat atria is preceded by a decrease in PIP_2 and an increase in IP_3. Whereas the decrease in PIP_2 and the increase in IP_3 could already be detected at 30 s, the increase in force of contraction did not start before 1 min. In rat papillary muscles, Otani et al. (1988) showed that concentration-response curves for α_1-adrenoceptor-mediated [^{3}H]inositol phosphate formation and inotropic responses were similar. In accordance with the view that the breakdown of PI may be involved in the positive inotropic effect of α_1-agonists is the finding that lithium, an inhibitor of inositol phosphate hydrolysis, potentiates the positive inotropic effect of α_1-adrenoceptor agonists (Molderings and Schümann, 1987; Skomedal et al., 1991).

If the hydrolysis of PIP_2 is an essential link in the pharmacomechanical coupling that follows the binding of the agonist to cardiac α_1-adrenoceptors, then the inhibition of PIP_2 hydrolysis should block the inotropic effect of α_1-adrenoceptor agonists. To test this hypothesis, Otani et al. (1988) exposed papillary muscles labeled with [^{3}H]inositol to 0.1 mM neomycin, a blocker of PIP_2 degradation. Neomycin inhibited [^{3}H]inositol phosphate formation and diminished the inotropic effects normally induced by α_1-adrenoceptor agonists. Wald et al. (1988) showed that another inhibitor of phospholipase C, 2-nitro-4-carboxyphenyl-N,N diphenylcarbamate, also inhibits the positive inotropic effect of the α_1-adrenoceptor agonist, methoxamine, in rat ventricular strips. However, neither neomycin nor 2-nitro-4-carboxyphenyl-N,N diphenylcarbamate are specific inhibitors of phospholipase C.

In rat papillary muscle, α_1-adrenoceptor stimulation produces a triphasic inotropic response. To investigate the specific role of the two limbs of the PI turnover (IP_3 and DAG-PKC) in the α_1-adrenoceptor-mediated positive inotropic effect, Otani et al. (1988) used 2,3-diphosphoglyceric acid which inhibits IP_3 degradation; this molecule potentiated the α_1-adrenergic mediated initial phases (the transient positive and negative phases) but had no effect on the sustained positive inotropic response. These authors concluded that PIP_2 degradation could play a role in the early inotropic response to α_1-adrenergic stimulation but that enhanced IP_3 formation cannot explain the sustained positive inotropic response.

There is some controversy as to whether or not IP_3 can release Ca^{2+} from the sarcoplasmic reticulum of cardiac muscle cells. Studying saponin-skinned myocytes and isolated sarcoplasmic reticulum, Movsesian et al. (1986) found no evidence that IP_3 (at concentrations up to 50 μM) can release Ca^{2+} from the sarcoplasmic reticulum. In saponin-skinned guinea pig papillary muscles, Nosek et al. (1986) demonstrated that Ca^{2+}-induced force oscillations are enhanced, in magnitude and frequency, by IP_3 at concentrations as low as 1 μM. IP_3 also increased the magnitude of caffeine contractures, caffeine being a strong releaser of Ca^{2+} from the sarcoplasmic reticulum. In mechanically skinned cardiac cells, Fabiato (1986) showed that 1,4,5-IP_3 induces a slow release of Ca^{2+} which causes a tension transient. This tension transient increased from 3 to 15% of maximum tension when the concentration of 1,4,5-IP_3 was increased from 2 to 30 μM. This author suggested that 1,4,5-IP_3-induced release of Ca^{2+} may play a role in the modulation of Ca^{2+} release by hormones or pharmacological agents. Recently, Molina-Viamonte et al. (1990) explored whether phospholipase C (0.05 units/ml), when applied extracellularly, increased Ca^{2+} transients in isolated, paced Purkinje fibers. Extracellularly applied phospholipase C augmented intracellular IP_3, suggesting a relationship between IP_3 generation and the size of the intracellular Ca^{2+} transient in intact cardiac tissue. Kentish et al. (1990) using the caged compound technique showed that photorelease of IP_3 in rat cardiac ventricular trabeculae triggers the release of Ca^{2+} from the sarcoplasmic reticulum as indicated by the tension developed by the muscle following the flash impulse. However, the magnitude of developed tension was much smaller than that developed in response to flashes that trigger Ca^{2+}-induced Ca^{2+} release. It was concluded that IP_3 does not trigger, but rather modulates, Ca^{2+}-induced Ca^{2+} release. Similar

conclusions were drawn from saponin-skinned chick atrial muscle by Vites and Pappano (1990) who reported that the tension developed in response to 20 μM IP_3 (maximal effect) was half of the force amplitude recorded in response to caffeine. Recently, Zhu and Nosek (1991) investigated the effects of IP_3 on Ca^{2+} release from sarcoplasmic reticulum in skinned rat papillary muscle. Based on the notion that Ca^{2+}-induced Ca^{2+} release and spontaneous cyclic Ca^{2+} release were distinct mechanisms for Ca^{2+} release from the sarcoplasmic reticulum (Fabiato, 1985), these authors demonstrated that IP_3 facilitates the spontaneous Ca^{2+} release rather than the Ca^{2+}-induced Ca^{2+} release.

Even if IP_3 could modulate the mobilization of intracellular Ca^{2+}, the positive inotropic effect of α_1-adrenoceptor agonists has been dissociated, at least in some experimental conditions, from an increase in intracellular Ca^{2+} transients (Dirksen et al., 1991; Gambassi et al., 1992; Terzic et al., 1992a). Furthermore, α_1-adrenoceptor agonists are able to increase contractile force above the levels that can be achieved by stimulators of Ca^{2+} influx (Terzic and Vogel, 1991).

In skeletal muscle, IP_3 can modulate the apparent Ca^{2+} sensitivity of contractile proteins (Thieleczek and Heilmeyer, 1986). However, in rabbit papillary muscle, Nosek et al. (1990) failed to demonstrate any effect of 30 μM IP_3 on the Ca^{2+} sensitivity of myofilaments (cf. Scholz et al., 1992a). Pucéat et al., (1990) obtained similar results in single isolated chemically skinned rat ventricular cells, a model in which molecular diffusion is facilitated. In summary, it appears that IP_3 alone cannot mediate the sustained positive inotropic effect of α_1-adrenoceptor agonists. This conclusion is also supported by the observation that IP_3 is only transiently increased following α_1-adrenoceptor stimulation, whereas the positive inotropic effect is sustained.

In frog heart cells, the α_1-adrenergic response occurs without a change in the action potential overshoot and with a less pronounced lengthening of the action potential duration than during the β-adrenergic action. Based on the observation that caffeine prevents the α_1-adrenergic inotropic response, Niedergerke and Page (1989) proposed that adrenoceptors facilitate Ca^{2+} discharge from the sarcoplasmic reticulum without requiring an increase in the I_{Ca} or in the process of Ca^{2+}-induced Ca^{2+} release; such a facilitation would result from the formation of IP_3. However, it should be kept in mind that caffeine also increases myofibrillar responsiveness to Ca^{2+}, a phenomenon that could lead to a misleading conclusion.

c. EVIDENCE THAT α_1-ADRENOCEPTOR STIMULATION INCREASES THE MYOFIBRILLAR RESPONSIVENESS TO CA^{2+}. COULD THE DIACYLGLYCEROL LIMB PLAY A ROLE IN MEDIATING THE EFFECTS OF α_1-ADRENOCEPTOR AGONISTS ON CONTRACTILE FORCE AND MYOFIBRILLAR SENSITIZATION? Endoh and Blinks (1988) investigated the effects of sympathomimetic amines on Ca^{2+} transients and isometric contractions in isolated rabbit papillary muscles in which multiple superficial cells had been microinjected with the Ca^{2+}-sensitive bioluminescent protein aequorin. These authors found that the modest increase in Ca^{2+} transient produced by phenylephrine was associated with a prominent increase in twitch contractile force. For a given increase in the force of contraction, α_1-adrenoceptor stimulation induces a smaller change in the amplitude of the Ca^{2+} transient than did other positive inotropic interventions. That is, the relation between the force developed and the amplitude of the aequorin signal is much steeper when the force is increased by α_1-adrenergic stimulation than when it is altered by other positive inotropic interventions. The suggestion was that the α_1-adrenoceptor agonists should increase the myofibrillar Ca^{2+} sensitivity (Endoh and Blinks, 1988; Endoh, 1986).

Subsequently, Capogrossi et al. (1988) simultaneously measured cytosolic Ca^{2+} and twitch amplitude in Indo-1-loaded cardiomyocytes. For a given Ca^{2+} transient, α_1-adrenoceptor agonists increased, whereas β-adrenoceptor agonists decreased, twitch amplitude. These authors concluded that α_1- and β-adrenoceptor stimulation produce opposite effects on myofibrillar sensitivity to Ca^{2+} (Gambassi et al., 1992). Thus, the principle that α_1-adrenergic stimulation increases the responsiveness of myofibrils to Ca^{2+} was confirmed for single cardiac cells.

It also has been postulated that α_1-adrenoceptor agonists increase the myofibrillar response to Ca^{2+} based on the effects of α_1-agonists on the parameters of the contraction-relaxation cycle (see section V.A.1) which strikingly resemble those induced by an increase in the length of the sarcomeres. The latter has been associated with an enhancement of myofibrillar sensitivity to Ca^{2+} (Meulemans et al., 1990; Li and Rouleau, 1991).

Definite evidence that α_1-adrenoceptor agonists indeed produce a myofibrillar sensitization to Ca^{2+} ions was obtained by Pucéat et al (1990). A preparation of isolated chemically skinned cells was used, and the force developed by a single cell in response to various Ca^{2+}-containing solutions was measured. This protocol was used to establish a tension-pCa relationship in the absence or presence of phenylephrine pretreatment. When cells were preexposed to phenylephrine before skinning, the tension-pCa curve was significantly shifted toward the left. Thereby, it was demonstrated independently of measuring intracellular Ca^{2+} that phenylephrine increased the Ca^{2+} sensitivity of myofilaments. The pCa_{50}, which is increased following the treatment of the cells with α_1-adrenoceptor agonists, returned to control values when alkaline phosphatase was applied to skinned cells. Thus, contrary to β-adrenoceptor agonists, which are known to decrease the Ca^{2+} sensitivity of myofibrils (McClellan and Winegrad, 1978), α_1-adrenoceptor ago-

nists are "sensitizing" cardiotonic agents (Pucéat et al., 1990, 1992; Terzic et al., 1992a).

Some evidence suggests that PKC activators mimic receptor-mediated myofibrillar sensitization. Pucéat et al. (1990) demonstrated, in skinned rat myocardial cells, that the application of PKC cell-permeant activators prior to skinning increases the myofibrillar responsiveness to Ca^{2+}, as indicated by a leftward shift of the tension-pCa relationship. The leftward shift was also reversed by the application of alkaline phosphatase to the skinned cells. This would imply that PKC activation causes a phosphorylation of the contractile proteins, thereby producing the observed increase in myofibrillar Ca^{2+} responsiveness. It is important to note that the direct application of PKC, purified from bovine brain, to skinned fibers was ineffective in altering the myofibrillar responsiveness to Ca^{2+}, whereas the application of cAMP-dependent protein kinase decreased the myofilament response. Hence, PKC per se appeared not to directly cause the enhanced responsiveness of myofibrils. However, the authors could not totally exclude the hypothesis that the lack of effect of PKC could be related to the brain PKC preparation they used. Indeed, the PKC isozyme profile is different in brain and heart (for review, see Kikkawa et al., 1989). More specifically, the preparation used did not contain the minor Ca^{2+}-insensitive isoforms of the kinase. In harmony with such an hypothesis, Collins et al. (1992), using a PKC pseudosubstrate inhibitor, showed that a Ca^{2+}-independent isoform of PKC mediated the α_1-adrenoceptor-induced contraction in ferret aorta cells. Moreover, Khalil et al., (1992), using the same preparation, reported that the translocation of the Ca^{2+}-independent isozyme ϵ of PKC was involved in the Ca^{2+}-independent contraction induced by phenylephrine in this tissue. It was postulated that an additional protein kinase that may be activated by PKC could be responsible for the myofibrillar sensitization. MLC kinase could be this additional kinase. Indeed, this kinase which specifically phosphorylates MLC-2 (for review, see Barany and Barany, 1980) increases the Ca^{2+} sensitivity of cardiac myofilaments (Morano et al., 1985; Clément et al., 1992). The ability of PKC to enhance MLC kinase-induced Ca^{2+} sensitization of the myofilaments has been reported in skinned cardiomyocytes (Clément et al., 1992).

In addition to sensitizing the myofibrils via phosphorylation, α_1-adrenoceptor agonists also could conceivably augment myofibrillar responsiveness to Ca^{2+} through an intracellular alkalinization. An increase in pH_i produces a positive inotropic effect (Vaughan-Jones et al., 1987) which is, in part, due to an increase in myofibrillar Ca^{2+} sensitivity (Fabiato and Fabiato, 1978). Although pH_i is a major regulator of cardiac excitation-contraction coupling (Kurachi, 1982; Solaro et al., 1988; Orchard and Kentish, 1990), pharmacological modulation of pH_i has only recently been considered as a potential means by which cardiac contractility could be regulated (Terzic and Vogel, 1990; Krämer et al., 1991; Terzic et al., 1992a; Wang and Morgan, 1992). As described in section IV.B, α_1-adrenoceptor agonists elevate pH_i by activating the Na^+/H^+ antiport (Iwakura et al., 1990; Terzic et al., 1992a; Wallert and Fröhlich, 1992; Pucéat et al., 1993a).

Several findings indicate that the activity of the antiporter could participate in the positive inotropic effects of α_1-adrenoceptor agonists. First, inhibition of Na^+/H^+ exchange by selective blockers (e.g., hexamethylamiloride, ethylisopropylamiloride) inhibits the increase in contractile force produced by phenylephrine in multicellular (Terzic and Vogel, 1990, 1991; Otani et al., 1990) or unicellular cardiac preparations (Gambassi et al., 1992) by at least 50%. Similarly, ionic substitution of Na^+ with ions that cannot replace Na^+ in Na^+/H^+ exchange markedly reduces the positive inotropic action of phenylephrine. Specifically, it is known that lithium, but not choline, will exchange for H^+ via the Na^+/H^+ antiport; phenylephrine-induced positive inotropic effects in choline-substituted solutions averaged 37% of that in lithium-substituted solutions (Terzic and Vogel, 1991). Second, the time course and magnitude of the α_1-adrenoceptor-mediated alkalinization closely correlates to that of the positive inotropic effect (Terzic et al., l991, 1992a; Gambassi et al., 1992). Third, the degree of alkalinization (0.1 pH unit) caused by α_1-adrenoceptor agonists (Terzic et al., 1992a) is known to increase contractile force by several-fold in cardiac tissue (Vaughan-Jones et al., 1987; Bountra and Vaughan-Jones, 1989; Lagadic-Gossmann and Feuvray, 1990).

Although PKC analogs may mimic some α_1-adrenergic effects, Yuan et al. (1987), Capogrossi et al. (1990), and Otani et al. (1992) showed that phorbol esters and 1,2-dioctanoylglycerol produce a negative inotropic response in perfused beating hearts, papillary muscle, or isolated rat ventricular myocytes. This result would not be predicted if PKC mediates the positive inotropic effect of α_1-agonists, unless an opposing effect of PKC activation was present in intact myocytes; this latter effect would lead to an overall negative inotropic effect. Indeed, in single cardiomyocytes loaded with the Ca^{2+} indicator Indo-1, phorbol-12-myristate-13-acetate and 1,2-dioctanoylglycerol markedly reduce the amplitude of the intracellular Ca^{2+} transient. This finding could explain why PKC activators produce a negative inotropic effect. Moreover, other groups have described a positive inotropic effect with 1,2-dioctanoylglycerol (10 to 100 μM) in electrically driven guinea pig atria (Teutsch et al., 1987) and rat cardiac myocytes (McLeod and Harding, 1991) or no effect of phorbol esters on contractile force in rabbit (Kushida et al., 1988) or rat papillary muscles (Otani et al., 1988). Whether the application of a PKC activator results in a positive, negative, or no inotropic effect may depend on the net effect of the intracellular pH change, the state of phosphorylation of the myo-

fibrils, the size of the intracellular Ca^{2+} transient (related or not to the external Ca^{2+} concentration), the state of Ca^{2+} loading and cellular tolerance to Ca^{2+}, and other unknown factor(s).

There have been contradictory reports regarding the ability of PKC blockers to prevent the positive inotropic effect of α_1-adrenoceptor agonists. On one hand, Otani et al. (1988, 1992) reported that staurosporine and H7 inhibited the sustained positive inotropic effect induced by α_1-adrenoceptor agonists in rat papillary muscles. On the other hand, Endou et al. (1991) showed that H7 does not affect the contractile response of rat papillary muscle to phenylephrine, and that neither phorbol 12,13-dibutyrate or 12-O-tetradecanoylphorbol-13-acetate reproduced the effects of α_1-adrenergic stimulation. Hence, there are discrepancies in the findings which, in part, depend on whether PKC was activated through the adrenoceptor or phorbol esters. It should be kept in mind that the diacylglycerol pathway represents only one limb of the PI signal transduction system and that receptor activation may generate additional cofactors. Furthermore, exogenously applied PKC activators may stimulate specific isozymes of PKC (Ryves et al., 1991; Otani et al., 1992; Pucéat et al., 1993b) that are not activated following receptor occupation. Non-PKC-dependent actions of phorbol esters cannot be excluded either (Watson and Karmazyn, 1991).

In summary, activators of PKC can increase myofibrillar Ca^{2+} sensitivity but do not always mimic the positive inotropic effects of α_1-adrenoceptor agonists. It is not clear whether there are other intracellular messengers, in addition to PKC, that mediate the inotropic effects of α_1-adrenoceptor agonists or whether the pharmacological tools used are imperfect. Thus, it may be premature to draw any definite conclusion regarding the role of the PI pathway in the positive inotropic effects of α_1-adrenoceptor agonists.

Unlike traditional "Ca^{2+} sensitizers" which directly bind to contractile proteins, α_1-adrenoceptor agonists increase the responsiveness of myofilaments to Ca^{2+} via two receptor-mediated mechanisms: (*a*) intracellular alkalinization and (*b*) phosphorylation of contractile protein(s). As previously argued (Terzic et al., 1992a), the leftward shift of the pCa-tension curve produced by the phosphorylation of the contractile protein(s) reaches 0.13 unit·pCa (Pucéat et al., 1990), whereas the shift expected from the alkalinization (approximately 0.1 unit·pH) can be calculated to amount to 0.07 unit·pCa (Fabiato and Fabiato, 1978). The phosphorylation of contractile protein(s) is not dependent on the alkalinization because it is not affected by Na^+/H^+ antiport inhibitors which completely abolish the intracellular increase in pH_i (Terzic et al., 1992a; reviewed by Pucéat et al., 1992). Consequently, a total pCa_{50} shift of 0.2 unit·pCa could be expected following α_1-adrenoceptor stimulation in vivo. Such a degree of Ca^{2+} sensitization of the myofilaments could account for a major portion of the α_1-adrenoceptor-mediated positive inotropic effect, albeit other mechanisms (e.g., prolongation of the action potential) could also be important (fig. 1). The intracellular balance between phosphorylation/dephosphorylation and alkalinization/buffer capacity may determine the respective importance of the two sensitizing mechanisms in the overall positive inotropic effect of α_1-adrenoceptor agonists. In addition, the intracellular control of the degree of phosphorylation and alkalinization produced by α_1-adrenoceptor agonists may prevent an oversensitization of the myofilaments to Ca^{2+}, an undesirable effect often observed with conventional Ca^{2+} sensitizers that directly bind to contractile proteins.

B. Chronotropic Effects

Usually, in normal adult hearts, α_1-adrenoceptor agonists induce no chronotropic action (Wagner and Brodde, 1978; Osnes et al., 1985). Thus, in contrast with many other positive inotropic drugs, such as β-adrenoceptor agonists, that produce cardiac acceleration, α_1-adrenoceptor agonists produce a positive inotropic effect without concomitant tachycardia. This result is at first surprising knowing that α_1-adrenoceptor stimulation modulates several ionic currents present in cardiac cells.

Cardiac rhythm is driven by pacemaker cells localized in specific areas of cardiac muscle, the sinoatrial and atrioventricular nodes. These cells are characterized by spontaneous depolarizations. The lack of an effect of α_1-adrenoceptor agonists on heart rate is probably due to the fact that these agents do not alter the pacemaker rate of the sinoatrial node (Hewett and Rosen, 1985). Although α_1-adrenoceptor agonists do not change the nodal rhythm, they do modulate the automaticity of latent pacemaker cells, such as isolated Purkinje fibers (for review, see Rosen et al., 1989). The α_1-adrenoceptor-induced modulation of rhythm apparently depends on the transmembrane potential because, when Purkinje fibers are depolarized to membrane potentials similar to those normally found in cells of the sinoatrial node, α_1-adrenergic stimulation loses its ability to modulate the automaticity of Purkinje fibers (Hewett and Rosen, 1985; Rosen and Robinson, 1990).

α_1-Adrenergic agonists increase or decrease the automaticity of isolated (normally polarized) Purkinje fibers, depending on the stage of development and on the specific subset of fibers. The majority of adult Purkinje fibers exposed to phenylephrine exhibit a decrease in spontaneous firing rate. By contrast, α_1-adrenoceptor stimulation in immature Purkinje fibers usually produces an increase in automaticity. When neonatal rat cardiac myocytes were cocultured with sympathetic ganglionic cells, α_1-adrenoceptor agonists produced a negative chronotropic effect rather than the usual positive chronotropic action. Thus, the presence of sympathetic innervation may be critical to the development of the adult-

like chronotropic response to α_1-adrenoceptor agonists (Malfatto et al., 1990; for review, see Rosen et al., 1989, 1991).

In the adult postinnervated heart tissue, a pertussis toxin-sensitive 41-kDa G-protein links the α_1-adrenoceptor to negative chronotropy through a mechanism that involves activation of the Na^+/K^+-ATPase (Steinberg et al., 1985; Shah et al., 1988; Rosen et al., 1989). In the newborn heart, the α_1-adrenoceptor is coupled to positive chronotropy via a pertussis toxin-insensitive G-protein (Han et al., 1989). The acquisition of the pertussis toxin-sensitive G-protein depends on the maturation of the sympathetic innervation. This provides an explanation for the ontogenic change in the α_1-adrenergic effects on the chronotropic response from excitation (in newborn) to inhibition (in adult) (Drugge et al., 1985; Rosen et al., 1991). Neuropeptide Y, which is simultaneously released with norepinephrine from the sympathetic nerve endings, is probably responsible for the expression of the pertussis toxin-sensitive G-protein (reviewed by Rosen and Robinson, 1990) and, thus, could be the mediator of the change in chronotropic response from positive in neonates to negative in adults (Sun et al., 1991).

Both types of responses to α_1-adrenoceptor agonists are blocked by the α_1-adrenoceptor antagonist, prazosin. In addition, the decrease in automaticity is blocked by CEC, an α_{1B}-selective antagonist, whereas the increase in automaticity is antagonized by the α_{1A}-blocker, WB-4101 (del Balzo et al., 1990). These findings suggest that specific receptor subtypes may modulate different responses (Rosen et al., 1991).

Evidence has been gathered to link the α_{1B}-receptor to stimulation of the Na^+/K^+ pump, generation of a net outward current, and suppression of automaticity (Rosen et al., 1989; Shah et al., 1988; Zaza et al., 1990). The relationship between α_{1A}-receptor stimulation which triggers the increase in automaticity and PI hydrolysis also has been established. However, the role of the PI system in the increase of cardiac automaticity is still unclear (del Balzo et al., 1990; Molina-Viamonte et al., 1990; Rosen et al., 1991).

C. Arrhythmogenic and Other Detrimental Effects

α_1-Adrenergic mechanisms not only influence the automaticity of latent pacemakers but also play a role in the genesis of specific arrhythmias (Sheridan, 1986). Evidence has been obtained to implicate α_1-adrenoceptors, at least in some species, in the arrhythmias that occur during coronary artery occlusion and reperfusion (for review, see Benfey, 1987; Kurtz et al., 1991). α_1-Adrenoceptor blockade reduces the number of premature ventricular complexes during coronary reperfusion, reduces or abolishes ventricular tachycardia and fibrillation, and prevents the increase in idioventricular rate seen with coronary reperfusion (Sheridan et al., 1980; Penny et al., 1985; Culling et al., 1987). Conversely, α_1-adrenoceptor agonists increase idioventricular rate early after reperfusion in animals depleted of myocardial catecholamines (Sheridan et al., 1980). The enhanced α_1-adrenergic responsiveness is associated with a reversible increase in the number of myocardial α_1-adrenoceptors (Corr et al., 1981; Heathers et al., 1987; Dillon et al., 1988; Kurtz et al., 1991). However, α_1-adrenoceptors are not consistently elevated in all experimental models of myocardial ischemia (Dillon et al., 1988; Chess-Williams et al., 1990; Steinberg and Alter, 1993).

The α_1-adrenoceptor-triggered delayed afterdepolarizations were often observed in Ca^{2+}-overloaded Purkinje fibers during ischemia when O_2 availability is severely decreased (Kimura et al., 1984; Boutjdir and El-Sheriff, 1991). In contrast, in normoxic cardiomyocytes or Purkinje fibers, α_1-adrenergic stimulation failed to induce either early or delayed afterdepolarizations (Priori and Corr, 1990; Marchi et al., 1991) even though it decreased the threshold for ventricular fibrillation (Thandroyen et al., 1987). During reperfusion, α_1-adrenoceptor stimulation, by activating the Na^+/H^+ antiport, could be expected to increase the intracellular Na^+ concentration. In turn, an increase in intracellular Na^+ could lead to Ca^{2+} overload by a net uptake of Ca^{2+} via the Na^+/Ca^{2+} exchange. Although this cascade of events might be responsible for arrhythmias (Dennis et al., 1990), α_1-adrenoceptor agonists have not been shown to increase intracellular Na^+ unless the Na^+/K^+ pump is inhibited (Zaza et al., 1990; Terzic et al., 1991).

Automatic arrhythmias, as well as induced delayed afterdepolarizations and triggered activity, produced by α_1-adrenoceptor agonists in simulated ischemic conditions, are significantly reduced by WB-4101, a rather selective α_{1A}-antagonist (Anyukhovsky and Rosen, 1991; Molina-Viamonte et al., 1991). These results emphasize the role of a WB-4101-sensitive receptor subtype in ischemic arrhythmias and the potential antiarrhythmic ability for α_1-receptor subtype-selective blockade (Rosen et al., 1991). The increase in abnormal automaticity in ischemic Purkinje fibers depends on a WB-4101-sensitive α_1-adrenoceptor subtype whose actions are transduced by a pertussis toxin-sensitive 41-kDa G-protein and should be distinguished from the mechanism underlying the increase in automaticity in normal Purkinje fibers, which is independent of the pertussis toxin substrate (Anyukhovsky et al., 1992).

Stimulation of cardiac α_1-adrenoceptors hastens and potentiates the development of digitalis glycoside cardiotoxicity, as reported for isolated rat atria (Terzic and Vogel, 1990; Terzic et al., 1991). It has been proposed that the enhancement of digitalis cardiotoxicity is due to the stimulation of Na^+/H^+ exchange by α_1-adrenoceptor agonists. Indeed, the Na^+/H^+ antiport provides an important route of Na^+ loading (and, subsequently, Ca^{2+} loading) in conditions in which the Na^+/K^+ pump is blocked by toxic concentrations of digitalis (Frelin et al.,

1988; Kim and Smith, 1986; Kaila and Vaughan Jones, 1987). By stimulating the Na^+/H^+ antiport, α_1-agonists may aggravate digitalis-induced contractures by increasing both intracellular Na^+ and pH. In this regard, α_1-adrenoceptor agonists exert an opposite modulatory effect on ouabain cardiotoxicity when compared to Na^+/H^+ exchange blockers (Terzic et al., l991). The delayed afterdepolarizations induced by ouabain in canine Purkinje fibers are also worsened by α_1-stimulation (Lee and Rosen, 1993).

D. Induction of Gene Expression and Stimulation of Hypertrophy

Simpson (1983, 1985; for review, see Simpson et al., 1991) demonstrated that, in cultured neonatal rat cardiomyocytes, norepinephrine, via α_1-adrenoceptors, induces cell hypertrophy. Because cardiomyocytes which are highly differentiated are no longer able to divide, cardiac hypertrophy results primarily from an increase in protein content and, hence, in cell size. The hypertrophy produced by α_1-adrenergic stimulation is associated with an increase in myofibrillar protein synthesis without an effect on protein degradation (Meidell et al., 1986). Such an increase in protein content of cultured neonatal rat cardiac myocytes was inhibited by WB-4101, an α_{1A}-antagonist, to nearly the same extent as by prazosin a nonselective α_1-adrenergic antagonist. The α_{1A}-antagonist also inhibited the norepinephrine-induced increase in [^{3}H]inositol phosphates so that phosphoinositide phospholipase C seems to be involved in the "α_1-hypertrophic" response (Simpson et al., 1990). In contrast, CEC, the α_{1B}-antagonist, had no effect. The α_1-adrenoceptor-mediated stimulation of protein synthesis is blocked by selective Na^+/H^+ exchange inhibitors, suggesting the involvement of Na^+/H^+ exchange (Kagiya et al., 1992).

Following neurohormonal stimulation, cardiac hypertrophy proceeds through the following successive genetic events: (*a*) an immediate early gene expression of protooncogenes, such as c-*myc*, c-*fos*, *Egr1*, c-*jun*, and *jun-B*. This genetic program occurs within 1 to 2 h and does not require any protein synthesis. Most of these protooncogenes encode transcriptional factors (or related proteins that behave as transcriptional factors) that bind DNA and activate the transcription machinery; (*b*) a reactivation, within 24 h in neonatal cardiac cells, of the expression of embryonic genes such as ANP, skeletal α-actin, and β-myosin heavy-chain genes; (*c*) an up-regulation, within 24 and 48 h, of constitutively expressed contractile protein genes (MLC-2, cardiac α-actin). It should be pointed out that the time course of these genetic events can vary between different in vivo models of hypertrophy.

α_1-Adrenergic stimulation triggers most of these genetic events (table 2). More specifically, α_1-adrenoceptor agonists augment the expression of c-*myc*, a gene that is normally involved in cell proliferation and transformation (Starksen et al., 1986; Ikeda et al., 1991). The induction of c-*myc* expression in cultured cardiac myocytes is rapid (maximum reached within 1 to 2 h) and short-lived (by 6 h after stimulation, c-*myc* mRNA returns to control levels). The mechanism by which the α_1-adrenoceptor enhances c-*myc* expression is not known. The PKC activator, phorbol-12-myristate, also increases the levels of c-*myc* mRNA and produces hypertrophy in cultured cardiac myocytes (Starksen et al., 1986). α_1-Adrenoceptor agonists rapidly activate (within 15 to 30 min) the expression of two other protooncogenes, namely, c-*fos* and c-*jun*, and the inducible zinc finger gene, *Egr-1*. These genes are involved in the development of cell hypertrophy through a pertussis toxin-insensitive mechanism (Iwaki et al., 1990). Phorbol-12-myristate-13-acetate is also capable of inducing the expression of the protooncogenes c-*fos* and c-*jun* and the *Egr-1* gene (Dunnmon et al., 1990).

α_1-Adrenergic stimulation produces a several-fold increase in the number of sarcomere units in the cellular content of cardiac myofibrillar genes (MLC-2, skeletal and cardiac α-actin) and in the steady-state levels of the corresponding mRNA (Lee et al., 1988; Iwaki et al., 1990; Bishopric et al., 1987; Long et al., 1989). Because phenylephrine did not produce a similar effect in nonmyocardial cells, it was concluded that the α_1-adrenoceptor-mediated increase in transcription activity is specific for cardiac genes (Lee et al., 1988). The direct activation of PKC by phorbol-12-myristate-13-acetate also induces the expression of the MLC-2 gene and increases the accumulation of the contractile protein in neonatal cells (Dunnmon et al., 1990). These data suggest that PKC could participate in the α_1-adrenoceptor-induced expression of contractile protein genes.

α_1-Adrenoceptor agonists are also potent activators of the expression of the ANP gene (Knowlton et al., 1991). The α_1-adrenoceptor induced coexpression of the gene *Egr-1* could play a role in the expression of contractile proteins and ANP genes (Iwaki et al., 1990). PKC and Ca^{2+}-calmodulin-dependent kinases have been reported to be involved in the α_1-adrenoceptor-induced ANP gene expression (Sei et al., 1991). Shubeita et al. (1992) confirmed that phenylephrine induces the expression of ANP and MLC-2 genes. The α-agonist increased by 12- and 5-fold the accumulation of the ANP and MLC-2 mRNA, respectively. Moreover, using neonatal myocytes transfected with constructs containing the ANP or MLC-2 promoter associated with the luciferase cDNA, Shubeita et al. (1992) observed an increase in the luciferase activity in phenylephrine-stimulated cells. Using the same approach, these authors reported that cotransfection of vectors encoding constitutively active α- and/or β-isozymes of PKC also increased the luciferase activity. This may suggest that the α- and/or β-isoforms of PKC could be involved in the expression of ANP and MLC-2

TABLE 2
α_1-Adrenoceptor-mediated gene induction

Oncogenes	Contractile protein genes	Other genes
c-*myc*: Starksen et al. (1986), Ikeda et al. (1991)	MLC-2: Lee et al. (1988), Iwaki et al. (1990)	ANP: Knowlton et al. (1991)
c-*fos*, c-*jun*, *Egr1*: Iwaki et al. (1990)	β-Myosin heavy chain: Waspe et al. (1990) Skeletal α-actin: Bishopric et al. (1987) Cardiac α-actin: Long et al. (1989)	

genes induced by α-adrenoceptor agonists. The α_{1A}-adrenoceptor-selective antagonist, (+)-niguldipine, inhibits the transcriptional activation of the ANP-luciferase fusion gene. This suggests that cardiac α_{1A} may be involved in the induction of embryonic gene expression in neonatal cells (Michel et al., 1990), which is in agreement with the finding that the α_{1A}-adrenoceptor mediates cell hypertrophy (Simpson et al. 1990). The expression of these embryonic genes occurs following a 24- to 48-h α_1-adrenergic stimulation of neonatal cardiomyocytes. α_1-Adrenoceptor stimulation was recently shown to up regulate β-myosin heavy chain iso-mRNA (Waspe et al., 1990), probably through the stimulation of the β-isozyme of PKC (Kariya et al., 1991).

In adult ventricular cells, α-adrenoceptor agonists induce the expression of the 15-kDa protein "Id" (for "inhibitor of DNA binding") (Springhorn et al., 1992). This protein prevents the binding to DNA of muscle potentiators of differentiation, such as myoD, myogenin, and Mif5 (Benezra et al., 1990). These molecules serve as tissue-specific transcriptional factors. It should be pointed out that, although these factors are not expressed in the heart, it is likely that similar factors, not yet identified, are responsible for the cardiac phenotype (for review, see Olson, 1993). The expression level is usually high in undifferentiated proliferating cells but diminishes with growth arrest and when cells begin to differentiate. Concomitantly with the induction of Id, α-agonists increased by 51% the rate of protein synthesis (Springhorn et al., 1992). This study raises the possibility that, in response to physiological stimuli, including catecholamines' action through α-adrenoceptors, Id could modulate cell growth and regulate the cardiac phenotype's plasticity both during cardiac ontogeny and in the adult.

Myocardial hypertrophy is an adaptive response of the heart to hemodynamic overload and commonly occurs in patients with hypertension and valvular heart disease (Swynghedauw and Delcayre, 1982). Zierhut and Zimmer (1989) reported that the intravenous infusion of norepinephrine for 3 days triggered the development of left ventricular hypertrophy as indicated by changes in several functional parameters (e.g., increase in heart rate and left ventricular rate of change of pressure and increased total peripheral resistance), as well as increases in the RNA to DNA and left ventricle weight to body weight ratios. The authors attributed this effect to both β- and α_1-adrenoceptor stimulation. In response to cardiac overload, the density of cardiac α_1-adrenoceptors was enhanced and preceded the development of cardiac hypertrophy in pressure-overloaded hearts (Tamai et al., 1989). An enhanced α_1-adrenoceptor activity and an excessive α_1-adrenoceptor-mediated growth may subserve protein synthesis in response to pressure overload. More recently, Kagiya et al. (1991a) showed that, in cardiomyopathic hamsters, α_1-adrenoceptor density remained higher than in control animals during the development of the hypertrophic stage; the authors also observed an attenuation of the hypertrophy when α_1-adrenoceptors were blocked. They, thus, concluded that α_1-adrenergic stimulation played an important role in the progression of cardiac hypertrophy in cardiomyopathy.

VI. Existence of Functional α_1-Adrenoceptors in Human Cardiac Tissue

A. In Vitro Studies

Human cardiac cells possess α_1-adrenoceptors. This has been demonstrated by binding studies using selective α_1-adrenoceptors ligands, [^{3}H]prazosin or [^{125}I]IBE 2254 (Bevilacqua et al., 1987; Böhm et al., 1988b; Bristow et al., 1988; Steinfath et al., 1992a,b). In the presence of GTP, a rightward shift of the displacement curve for unlabeled α_1-agonists occurred, suggesting that human cardiac α_1-adrenoceptors are linked to a GTP-binding protein (Bevilacqua et al., 1987). It is not yet known which α_1-adrenoceptor subtypes are present in human myocardial cells. The gene encoding the human α_{1B}-adrenoceptor has been cloned (Ramarao et al., 1992). The nucleotide sequence predicts a seven-transmembrane domain receptor made of 517 amino acids and with a molecular mass of 57 kDa. A high homology exists between this human receptor and the α_{1B}-adrenoceptor found in rat, hamster, and dog. The α_{1B}-adrenoceptor gene is transcribed in human hearts as demonstrated by Northern blot analysis with the aid of a fragment from a heart cDNA library that corresponds to exon 1 of the gene (Ramarao et al., 1992).

The stimulation of human atrial or ventricular α_1-adrenoceptors by endogenous catecholamines or synthetic sympathomimetics, in the presence of β-adrenoceptor blockade, produces a positive inotropic effect (Schümann et al., 1978; Wagner et al., 1980; Brückner et al., 1984; Skomedal et al., 1985; Aass et al., 1986; Ask et al., 1987; Böhm et al., 1988b; Kohl et al., 1989; Jahnel et al., 1992a). The magnitude of the increase in twitch

contractile force produced by α_1-adrenergic agonists varies among studies. Indeed, the responsiveness of the human cardiac tissue to α_1-adrenoceptor stimulation can be affected by several factors. These include the prior condition of the heart (e.g., failing versus nonfailing), exposure of cardiac muscle to different drugs, and the procedure of tissue removal during surgery and further manipulations of the specimens. In nonfailing human hearts α_1-adrenoceptor stimulation can increase the force of contraction more than 2-fold (Kohl et al., 1989; Terzic, 1990). In failing human hearts, the α_1-mediated positive inotropic effect is usually smaller (Schmitz et al., 1987a; Böhm et al., 1988b; Jakob et al., 1988; Steinfath et al., 1992b). The mechanism responsible for the decrease in inotropic responsiveness to α_1-adrenergic agonists with the progression of heart failure is not known.

The absolute number of α_1-adrenoceptors does not change or even increase during the development of cardiac failure (Bristow et al., 1988; Steinfath et al., 1992b). In cardiac membranes obtained from patients with end-stage heart failure (New York Heart Association IV, due to an idiopathic dilated cardiomyopathy), the density of ventricular α_1-adrenoceptors, assessed using [^{3}H]prazosin, was found to be 11 fmol/mg protein (nonfailing hearts, 4 fmol/mg protein) (Steinfath et al., 1992a,b). Because there is no reduction of cardiac α-adrenoceptors but an increased ratio of α to β adrenoceptors, α-adrenoceptors might contribute to the maintenance of cardiac contractility in heart failure, in which β-adrenoceptors-mediated responses are severely compromised (Bristow et al., 1982; Böhm et al., 1988b).

The mechanism of the positive inotropic effect of α_1-adrenergic agonists in human tissue is a matter of current investigation. In atrial tissue, the positive inotropic effect is not accompanied by an increase in the action potential duration but rather by a decrease (Jahnel et al., 1992a). On the other hand, in a preliminary study performed using the whole cell patch clamp technique in single atrial cells isolated from nonfailing hearts, methoxamine, in the presence of propranolol, reduced the transient outward current independent of Ca^{2+}, an effect that might favor a prolongation of the action potential (B. Legrand and E. Coraboeuf, personal communication).

In human ventricular trabeculae, phenylephrine produces an enhanced breakdown of PIP_2 and phosphatidylinositol phosphate. Accordingly, IP_3 and its congeners IP_2 and IP_1 are increased (Kohl et al., 1989).

B. In Vivo Studies

Attempts to demonstrate the effects of α_1-adrenoceptor agonists and antagonists on myocardial contractility in vivo in humans are hampered by the confounding effects of stimulation and inhibition of vascular α_1-adrenoceptors on ventricular loading conditions and reflex mechanisms (Curiel et al., 1989). To avoid the systemic effects of α_1-adrenoceptor stimulation or inhibition, Landzberg et al. (1991) infused α_1-adrenoceptor agonists or antagonists into the left main coronary artery of subjects with normal left ventricular function. Intracoronary infusion of phenylephrine caused an increase in the peak rate of left ventricular pressure increase, which is known to provide a reliable index of changes in inotropic state under these conditions (Colucci, 1990). The concurrent infusion of phentolamine significantly reduced the response to phenylephrine (Landzberg et al., 1991). Although α_1-adrenoceptor stimulation increased contractility, the intracoronary infusion of the α_1-adrenoceptor antagonist, phentolamine, did not affect the baseline peak rate of left ventricular pressure increase. The authors concluded that endogenous myocardial α_1-adrenergic tone may not play a role in maintaining the basal state of left ventricular contractility in humans, at least when subjects rest in the supine position (Landzberg et al., 1991).

Consistent with the in vitro studies, the α_1-adrenoceptor-mediated positive inotropic effect is reduced in congestive heart failure (Landzberg et al., 1991). A reduction of α_1-adrenergic responsiveness without a reduction in α_1-adrenoceptor density (Bristow et al., 1988) might either reflect reduced efficiency of receptor coupling or be due to a cause not specifically related to α_1-adrenoceptors.

The demonstration that myocardial α_1-adrenoceptor are functional and capable of increasing myocardial contractility in humans may also have implications for other actions related to the myocardial α_1-adrenoceptor, namely, the modulation of gene expression, myocardial hypertrophy, recovery from intracellular acidosis during ischemia, and arrhythmias.

VII. Concluding Remarks

In this overview, we have attempted to summarize the remarkable progress that has been made in recent years toward understanding the function of cardiac α_1-adrenoceptors. However, many unresolved issues regarding the α_1-adrenoceptor-mediated regulation of myocardial function remain to be addressed. Although the stimulation of cardiac α_1-adrenoceptors produces a variety of cellular effects, especially during concomitant β-adrenoceptor blockade, it is still unknown under which conditions α_1-adrenoceptors play a major role in the adrenergic modulation of the heart.

The most studied acute α_1-adrenergic effect in cardiac preparations is the increase in twitch contractile force. Further investigation is required to quantify the extent to which different proposed inotropic mechanisms participate in the overall positive inotropic effect. An increase in the responsiveness of myofibrils to Ca^{2+}, subsequent to phosphorylation of contractile proteins and cytosolic alkalinization, appears to be important because, contrary to β-adrenoceptors, α_1-adrenoceptors mediate a positive inotropic effect without a marked change in the

intracellular Ca^{2+} concentration. In addition, the inhibition of the I_{to}, as well as the modulation of other conductances that lead to an action potential prolongation, could also contribute to the positive inotropic mechanism.

The nature of α_1-adrenoceptor subtypes and the subcellular pathways that transduce their signal need to be further elucidated. Although several α_1-adrenoceptor subtypes have been identified, their physiological significance and their exact relationship to specific cellular effects is still to be uncovered. Moreover, although it is established that the stimulation of α_1-adrenoceptors activates the turnover of PI through a G-protein, the nature of this regulatory protein is unknown. Furthermore, the phospholipase C isoenzyme(s) involved in the transduction cascade remain(s) to be elucidated. In addition to the PI metabolism, α_1-adrenergic stimulation could activate, at least under some circumstances, a cAMP-phosphodiesterase, a Ca^{2+}-calmodulin-dependent kinase, and phospholipases A_2 and/or phospholipase D. These two last enzymes could produce DAG from several sources, which in turn could either activate specific PKC isozymes or give rise to additional second messengers such as leukotrienes, prostaglandins, or cyclic guanosine monophosphate. A plethora of putative second messengers could be involved in mediating α_1-effects and thereby permit a fine regulation of cardiac function.

Most of the investigations related to α_1-adrenoceptors have been performed on single cardiomyocytes, isolated atria, ventricles, or perfused hearts. In some preparations the lack of consistent reproducibility has been reported with regard to α_1-adrenergic effects on I_{Ca} (Alvarez et al., 1987), Na^+/K^+ pump activation (Ertl et al., 1991), intracellular Ca^{2+} (Failli et al., 1992; Gambassi et al., 1992), or contraction (Niedergerke and Page, 1981). The origin of this variability is unknown, but it might be compared to the weakening of α_1-adrenergic effects in the failing heart (Schmitz et al., 1987a), their variations during development (Rosen et al., 1989), or the presence of the endocardial endothelium (Meulemans et al., 1990). In addition, synthetic α_1-sympathomimetics have been used in these investigations more commonly than the physiological neurotransmitter norepinephrine. Although markedly contributing to the understanding of α_1-effects in vitro, these studies have not uncovered the role of the α_1-adrenoceptor in vivo, because the cardiac preparations used were devoid of systemic regulatory mechanisms (e.g., concomitant β-adrenergic and other neurohormonal stimulations, cardiovascular reflexes) which interfere with the response of cardiac muscle to α_1-adrenoceptor stimulation. In this regard, Guse et al. (1991) recently reported that a simultaneous β-adrenergic stimulation strongly decreases the α_1-adrenoceptor-induced increase in inositol phosphates through a mechanism that remains to be determined.

Finally, most of these studies do not take into account the information provided by molecular biology studies. Indeed, chronic stimulation of cardiac muscle with α_1-adrenoceptor agonists modifies the expression of specific genes and could alter in a quantitative or qualitative manner several of the same cellular proteins that are targets of α_1-adrenergic action also on a short time basis.

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