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α_1-Adrenergic Receptor Subtypes, Inositol Phosphates, and Sources of Cell Ca^{2+}*

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I. Introduction

α_1-ADRENERGIC receptors are involved in a variety of important physiological processes, including control of blood pressure, appetite, and mood. These receptors belong to the class of cell-surface receptors which initiate signals in their target cells by increasing the concentration of free cytosolic Ca^{2+} and thereby affecting the metabolic or contractile state of the cell. Recent advances in our understanding of membrane lipid biochemistry have led to the conclusion that these receptors control cytosolic Ca^{2+} primarily by stimulating hydrolysis of a highly phosphorylated inositol phospholipid. The product of this hydrolysis, inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], has been shown to release Ca^{2+} sequestered in intracellular stores, particularly the endoplasmic reticulum.

Recent evidence suggests, however, that α_1-adrenergic receptors do not have the same properties in all tissues. The existence of distinct subtypes of α_1-adrenergic receptors has been supported by a variety of pharmacological approaches. Interestingly, it has also become clear from studies in smooth muscle that there are major differences in the importance of extracellular Ca^{2+} in responses to α_1-adrenergic receptor stimulation. In some tissues stored intracellular Ca^{2+} is sufficient for normal responses, while other tissues require influx of Ca^{2+} through specific membrane channels. Recent studies have raised the possibility that, in addition to the well-

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known α_1-adrenergic receptor controlling Ins(1,4,5)P_3 formation and release of intracellular Ca^{2+}, there is another pharmacologically distinct α_1-adrenergic receptor subtype controlling influx of Ca^{2+} through specific membrane channels. This subtype, which appears to be independent of inositol phospholipid metabolism, may be an example of a receptor-operated channel (34).

In this section I have attempted to summarize the pharmacological properties of α_1-adrenergic receptors and the mechanism(s) by which they initiate signals in target cells. I have concentrated most heavily on the pharmacological heterogeneity which has been observed between α_1-adrenergic receptors in different tissues and attempted to relate these pharmacological differences to the source of activator Ca^{2+} for tissue responses. Finally, I have surveyed the possible alternative mechanisms, in addition to formation of Ins(1,4,5)P_3, which might be important in transduction of signals by a novel α_1-adrenergic receptor subtype.

II. Adrenergic Receptor Subtypes

In 1948, R. P. Ahlquist (3) began the modern era in adrenergic pharmacology by recognizing that responses to adrenergic stimuli could be divided into two major categories. He proposed that the two major receptor subtypes, which he named α and β-, should be classified on the basis of their pharmacological properties rather than on the type of tissue response caused by receptor stimulation. Each receptor subtype could be excitatory in some tissues and inhibitory in others, but it was the ability of the receptors to recognize and respond to certain drugs which uniquely characterized each subtype. It was this fundamental insight, that receptor classification should be based on the drug specificities of the receptor recognition site, that laid the groundwork for the important receptor subclassification and selective drug development which has occurred subsequently.

In the 1960s it became clear that β-adrenergic receptors in different tissues showed different pharmacological properties. Lands and coworkers proposed that these receptors be divided into two subclasses called β_1- and β_2-adrenergic receptors (195, 196). β_1-Adrenergic receptors were found predominantly in heart, while β_2-adrenergic receptors were found predominantly in smooth muscle. Recent evidence from radioligand binding assays supports this subclassification and has made it clear that these receptor subtypes can coexist in the same tissue and on the same cells (248). β-Adrenergic receptor subtypes show many similarities and only a few differences: both β_1 and β_2-adrenergic receptors stimulate formation of cyclic AMP as their primary mechanism for signal initiation in cells; and very few drugs show more than a 20- to 50-fold difference in potency in binding to the two different subtypes. However, the use of molecular biological techniques has demonstrated that the two receptor subtypes are not produced by alternative mRNA splicing or cell-specific posttranslational modifications, but that the properties of each subtype are intrinsic to the receptor gene products (328).

In a similar vein, during the 1970s it became clear that α-adrenergic receptors in different tissues do not have identical pharmacological properties. Based on differences in the potency of phenoxybenzamine in blocking presynaptic increases in norepinephrine release and postsynaptic increases in contractility in cat spleen (70), Langer (197) proposed that postsynaptic α-adrenergic receptors be referred to as α_1, and presynaptic receptors be referred to as α_2. Berthelsen and Pettinger (23) suggested that α_2-adrenergic receptors may have several nonneuronal effects and proposed that these receptors also be classified pharmacologically rather than on the basis of their anatomical localization. The subsequent realization that α_2- as well as α_1-adrenergic receptors exist on smooth muscle cells and activate contractile responses (90, 96, 199, 322, 339, 344) supported the importance of classifying these receptor subtypes on the basis of their drug specificities, rather than by where they are located or what type of response they subserve. It subsequently also became clear that α_1-adrenergic receptors could exist on presynaptic nerve terminals (89, 190, 225, 327), particularly regulating transmitter release from sympathetic nerves in rat heart.

It is remarkable that, although α-adrenergic receptors were subclassified almost a decade later than β-adrenergic receptors, the α-adrenergic receptor subtypes appear to be much less closely related than β-adrenergic receptor subtypes. Highly selective agonists and antagonists with substantially different affinities for α_1- and α_2-adrenergic receptors were discovered within a few years of the original subclassification (50, 323). Antagonist selectivities of two or three orders of magnitude between α_1- and α_2-adrenergic receptors are not uncommon, while selectivities of only one to two orders of magnitude are more commonly observed for β_1- and β_2-adrenergic receptors. Although β_1- and β_2-adrenergic receptors appear to share a common signal transduction mechanism, i.e., stimulation of cyclic AMP formation, α_1- and α_2-adrenergic receptors use completely different mechanisms for initiating signals in their target cells. α_1-Adrenergic receptors appear to control cytosolic Ca^{2+} levels through effects on inositol phospholipid metabolism, while α_2-adrenergic receptors inhibit adenylate cyclase and decrease cyclic AMP levels (103).

These observations raise the interesting possibility that each major α-adrenergic receptor subtype might encompass a family of more closely related subtypes. In this article I will discuss the evidence for the existence of subtypes of α_1-adrenergic receptors. It is interesting to note, however, that several lines of evidence now suggest that there are pharmacologically distinct subtypes of α_2-adrenergic receptors. Differences in the pharmacological properties of α_2-adrenergic receptors in different tissues have been reported (180, 258), and α_2-

adrenergic receptors have been shown to accelerate Na^+/H^+ exchange in addition to inhibiting adenylate cyclase activity (158). Finally, cloning of the gene for the α_2-adrenergic receptor in human platelets has revealed two related genes which hybridize under low stringency conditions, possibly coding for additional α_2-adrenergic receptor subtypes (189). It will be interesting, therefore, to examine more closely the properties of α-adrenergic receptors in different tissues.

III. α_1-Adrenergic Receptors

α_1-Adrenergic receptors are generally defined as receptors which are potently blocked by the competitive antagonists phentolamine and prazosin, irreversibly blocked by the alkylating agent phenoxybenzamine, and selectively stimulated by the agonists phenylephrine and methoxamine. In addition, a wide variety of other drugs are available to distinguish these receptors from other adrenergic receptor subtypes, including both competitive antagonists, alkylating agents, and agonists. Examples of such drugs mentioned in this review are given in table 1.

α_1-Adrenergic receptors are found in almost all mammalian tissues, but serve a particularly important function in smooth muscle. Norepinephrine released from sympathetic nerves acts on α_1-adrenergic receptors to increase cytosolic Ca^{2+} and promote smooth muscle contraction, and this appears to be one of the primary mechanisms by which the sympathetic nervous system controls total peripheral resistance (44). α_1-Adrenergic receptors are also probably very important in the central nervous system. While their precise function is still uncertain, there is a high density of this subtype in most brain regions (167), and specific agonists and antagonists have marked effects on membrane currents in neurons (1) and on animal behavior (9, 262). In addition, this receptor subtype is important in controlling the contractile status of nonvascular smooth muscles (44), and under certain physiological conditions it is responsible for mediating the effects of catecholamines on glycogen breakdown in liver (154).

TABLE 1

Drugs useful in classifying α-adrenergic receptor subtypes [see Berthelsen and Pettinger (23), Starke (322), and Timmermans and van Zwieten (344) for references].

	α_1 selective	α_2 selective	Nonselective
Antagonists	Prazosin BE 2254 WB 4101 Indoramin	Yohimbine Rauwolscine Idazoxan Piperoxan	Phentolamine Dihydroergotamine
Agonists	Phenylephrine Methoxamine Cirazoline 6-Fluoronorepinephrine	Clonidine UK 14,304 Guanabenz BHT 920	Norepinephrine Epinephrine
Alkylating agents	Phenoxybenzamine Dibenamine		EEDQ Benextramine

An intriguing question about α_1-adrenergic receptor function which has been receiving much recent attention is the possibility that this subtype may be involved in some of the effects of catecholamines on the heart. Although these effects are generally mediated by β-adrenergic receptors, it is now clear that α_1-adrenergic receptors exist in this tissue in some species in a density similar to or even greater than that of β-adrenergic receptors. Stimulation of these receptors causes a relatively slow positive inotropic response in many species, including man (18, 40, 317, 362), as well as a positive chronotropic response in rat heart (104, 347). Although the functional role of these receptors is obscure, they may function as a reserve mechanism for cardiac stimulation under various stressful or pathological conditions.

It is clear that α_1-adrenergic receptors play a variety of important roles in mammalian physiology. Similarly, agonists and antagonists selective for this receptor type have found widespread use in various clinical applications, including relieving nasal congestion, reducing local blood flow, inducing mydriasis for ophthalamic examinations, and in treatment of hypertension, hypertensive crisis, pheochromocytoma, and paroxysmal atrial tachycardia (143).

IV. Signal Transduction through Inositol Phospholipid Metabolism

α_1-Adrenergic receptors are among those receptors which utilize changes in intracellular free Ca^{2+} as their primary signal transduction mechanism. Recent rapid progress in this field has led to a detailed understanding of the mechanisms by which these receptors control the mobilization of stored intracellular Ca^{2+}. Like other "Ca^{2+}-mobilizing" receptors, activation of α_1-adrenergic receptors increases the hydrolysis of a specific membrane lipid to release diffusible second messenger substances into the cell cytosol.

For many years, numerous reports linked stimulation of α-adrenergic receptors to increases in turnover of the membrane lipid phosphatidylinositol (145, 172). Michell (237) proposed that all receptors which increase cytosolic Ca^{2+} levels also increase turnover of phosphatidylinositol, and proposed that this effect might be involved in the opening of Ca^{2+} gates. This hypothesis was strongly supported by the results of Fain and Berridge (102) who showed that phosphatidylinositol breakdown appeared to be intimately involved in the stimulation of fluid secretion by serotonin in an insect salivary gland. Their results made it clear that breakdown of phosphatidylinositol was probably an important pathway for the formation of second messengers involved in control of cytosolic Ca^{2+} levels, and Fain and Garcia-Sainz (103)

postulated that this was the major mechanism of signal transduction for α_1-adrenergic receptors.

Phosphatidylinositol is unique among membrane lipids in that it also occurs in two more highly phosphorylated forms, i.e., phosphatidylinositol-4-monophosphate (PIP) and phosphatidylinositol-4,5-bisphosphate (PIP_2). Further work, particularly by Berridge and colleagues, made it clear that stimulation of Ca^{2+}-mobilizing receptors causes activation of a membrane-bound phospholipase C to catalyze the breakdown primarily of PIP_2, and release diacylglycerol and $Ins(1,4,5)P_3$ (21). The formation of $Ins(1,4,5)P_3$ appears to link receptor activation to release of intracellular Ca^{2+}, since this compound was soon shown to cause release of Ca^{2+} from nonmitochondrial intracellular stores in a variety of cell types (174, 329). Interestingly, the diacylglycerol released also appears to serve a second messenger function; this compound activates protein kinase C by reducing its requirement for Ca^{2+} (261). Thus agonist-induced hydrolysis of PIP_2 generates two intracellular messengers, $Ins(1,4,5)P_3$, which mobilizes stored intracellular Ca^{2+}, and diacylglycerol, which activates phosphorylation of specific cellular proteins (20).

There is strong evidence that α_1-adrenergic receptor activation causes hydrolysis of inositol phospholipids in almost all tissues where this phenomenon has been looked for. The use of lithium to inhibit breakdown of inositol monophosphates by myo-inositolmonophosphatases (22, 127) has made it relatively easy to examine accumulation of [^{3}H]inositol phosphates in response to receptor activation. Increased accumulation of [^{3}H]inositol phosphates in response to α_1-adrenergic receptor activation in the presence of lithium has been demonstrated in parotid (22), brain (22, 42, 247, 306), liver (275), cultured neuronal and glial cells (122, 269), cardiac myocytes (43), smooth muscle (110, 360), and kidney (259) as well as other tissues. Although these reports indicate strong correlations between activation of α_1-adrenergic receptors and increased inositol phospholipid hydrolysis, it is important to remember that what was measured in most of these experiments was only production of total [^{3}H]inositol phosphates in the presence of lithium. Individual inositol phosphate species have not usually been separated or quantified. It is often assumed that the increase in inositol phosphates observed following α_1-adrenergic receptor stimulation is due primarily to PIP_2 hydrolysis and formation of $Ins(1,4,5)P_3$. However, this has actually been shown in only a few instances. In most cases the source of the inositol phosphates which accumulate in the presence of lithium has not yet been clearly identified.

Although there is widespread agreement that the two products of PIP_2 hydrolysis, $Ins(1,4,5)P_3$ and diacylglycerol, play important roles in mobilizing stored intracellular Ca^{2+} and activating protein kinase C, it is not clear whether these are the only second messengers involved in the actions of Ca^{2+}-mobilizing receptors. The situation has recently become more interesting with the realization that inositol phosphate metabolism is extremely complex, and other inositol phosphates have been identified which may perform important second messenger functions. Inositol 1-monophosphate [Ins(1)P], inositol 1,4-bisphosphate [$Ins(1,4)P_2$], and $Ins(1,4,5)P_3$ all exist in cyclic forms which are also made directly by phospholipase C attack on phospholipid precursors (221), and in some cases they may be as potent as the noncyclic forms in activating Ca^{2+} mobilization. In addition, $Ins(1,4,5)P_3$ is rapidly phosphorylated in many tissues to form inositol 1,3,4,5-tetrakisphosphate [$Ins(1,3,4,5)P_4$] (14, 155). The rapid formation of this compound raises the interesting possibility that it might also play a second messenger role, and it has been suggested that $Ins(1,3,4,5)P_4$ is involved in opening cell-surface Ca^{2+} channels (156). The existence of this compound explains the previously puzzling fact that inositol 1,3,4-trisphosphate [$Ins(1,3,4)P_3$], and not $Ins(1,4,5)P_3$, is in many cases the primary inositol trisphosphate formed following receptor stimulation (235). $Ins(1,3,4)P_3$ appears to be formed by dephosphorylation of $Ins(1,3,4,5)P_4$ (93). Interestingly, even more highly phosphorylated forms of inositol also exist in animal cells (12, 137), although no functional role has yet been proposed for these compounds. It is likely that a further understanding of the complicated metabolic pathways of these compounds will lead to further insights into the mechanisms of receptor-mediated signal transduction.

V. Involvement of Guanine Nucleotide Regulatory Proteins

The mechanism by which α_1-adrenergic receptor stimulation activates PIP_2 breakdown is not yet fully understood. However, in a manner analogous to receptor-mediated alterations in adenylate cyclase activity, it seems likely that, in at least some cases, a guanine nucleotide regulatory protein (G protein) is involved in activation of phospholipase C.

The first evidence for involvement of a G protein in the actions of α_1-adrenergic receptors came from studies of the effects of guanine nucleotides on the affinity constants of agonists in radioligand binding studies. It was well established that the binding affinity of agonists (but not antagonists) for receptors regulating adenylate cyclase activity through G proteins was markedly affected by GTP and its nonhydrolyzable analogs (207). This is due to formation of a stable high affinity ternary complex of agonist/receptor/G protein in the absence of GTP. This complex is destabilized by GTP to promote dissociation of the G protein, leaving only the relatively low affinity agonist/receptor complex. Since it was thought that only adenylate cyclase-linked receptors participated in such a GTP-modulated equilibrium, it was surprising when Goodhardt et al. (123) (fig. 1) and Snaveley and Insel (319) reported that guanine nucleotides

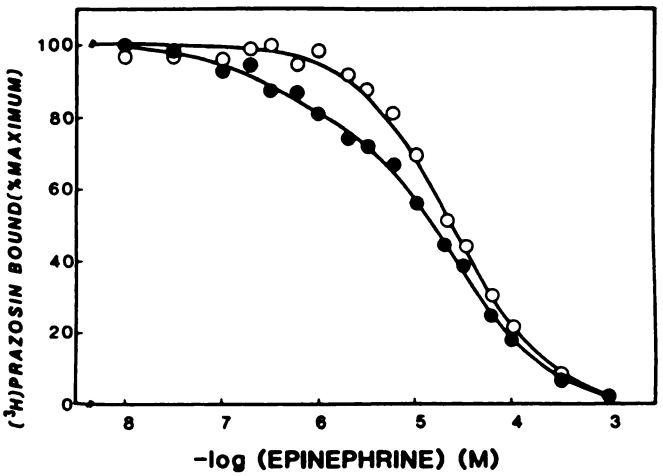


FIG. 1. Effect of the nonhydrolyzable GTP analog GppNHp on the potency of epinephrine in inhibiting specific [^{3}H]prazosin binding to rat liver plasma membranes. ●, assays carried out in the absence of GppNHp; ○, assays carried out in the presence of 0.1 mM GppNHp. Note the presence of both high and low affinity binding components for epinephrine in control tissues. The effect of the guanine nucleotide is to convert all receptors to the apparent low affinity state. From Goodhardt et al. (123) with permission.

also modulated the affinity constants of agonists at α_1-adrenergic receptors. This observation was subsequently confirmed by others (38, 66, 215) and was the first evidence that α_1-adrenergic receptors could interact with G proteins.

More direct evidence that G proteins are involved in receptor-mediated alterations in phospholipase C activity came from Gomperts (119) who showed that guanine nucleotides stimulated a Ca^{2+}-dependent secretion of histamine from permeabilized mast cells. Stable analogs of GTP have been shown to activate PIP_2 breakdown in permeabilized pancreatic acinar cells (236) and neutrophils (39), and also in membrane preparations from neutrophil (64), liver (363), brain (121), turkey erythrocytes (132), and other tissues (209). These results support the concept that G proteins regulate the activity of phospholipase C. In addition, direct activation of PIP_2 hydrolysis in membrane preparations has now been observed in response to activation of variety of receptors, including serotonergic (210), muscarinic cholinergic (134), α_1-adrenergic, vasopressin, and angiotensin (348). As expected, this activation is dependent on the presence of guanine nucleotides, supporting the involvement of a G protein.

The identity and properties of the G protein(s) involved are not certain. Pertussis toxin, which inactivates the G_i protein which is responsible for receptor-mediated inhibition of adenylate cyclase (207), blocks activation of inositol phospholipid breakdown stimulated by some receptor types (39, 257, 274), and blocks some of the effects of α_1-adrenergic receptor stimulation in cardiac myocytes cocultured with sympathetic neurons (324). Since pertussis toxin is thought to selectively ADP-ribosylate the G_i protein responsible for inhibiting adenylate cyclase (114), the G protein involved in these receptor-mediated alterations in inositol phospholipid hydrolysis may be similar to G_i. However, in most systems, pretreatment with pertussis toxin has no effect on receptor-mediated activation of phosphoinositide breakdown (46, 134, 209, 236). Why pertussis toxin should block receptor-mediated inositol phospholipid hydrolysis in some systems but not others is not yet clear. Possibly two different G proteins are involved in activation of phospholipase C by different receptors. Alternatively, alterations in inositol phospholipid hydrolysis could, in some cases, come subsequently to another signal which requires an intact G_i protein (i.e., inhibition of adenylate cyclase).

Free cytosolic Ca^{2+} is also involved in maintaining phospholipase C activity and PIP_2 hydrolysis. Although formation of Ins(1,4,5)P_3 is thought to release intracellular Ca^{2+}, the activation of PIP_2 hydrolysis by receptor stimulation is blocked by chelation of Ca^{2+} (120, 169, 184, 188), suggesting that activation of the enzyme is dependent on at least a minimal concentration of Ca^{2+} inside cells, although there is no evidence that this process is under the *control* of Ca^{2+}. In membrane preparations, Ca^{2+} has been shown to increase the activity of phospholipase C (121, 144), and depolarization has been shown to increase phosphoinositide hydrolysis in brain slices in a manner sensitive to inhibition by dihydropyridine-type Ca^{2+} entry blockers (124, 185, 220, 291) (fig. 2). Although such effects might be caused by a calcium-dependent release of neurotransmitters which stimulate inositol phospholipid metabolism, such release is usually not inhibited by dihydropyridine-type Ca^{2+} entry blockers (see below). Such data may be explained at least partially by the existence of two types of phosphatidylinositol-specific phospholipase C enzymes as shown by Hoffman and Majerus (144), although these enzymes were isolated from cytosolic, not membrane, compartments. The relationship of receptor-, G protein-, and Ca^{2+}-activated phospholipase C activity is not yet clear. The involvement of Ca^{2+} in inositol phospholipid hydrolysis is discussed in more detail below.

VI. Alternate Signal Transduction Mechanisms

Although α_1-adrenergic receptor activation increases inositol phospholipid hydrolysis in every tissue so far studied (see above), this may not be the only mechanism through which these receptors initiate signals in their target cells. Increasing evidence suggests that a variety of other mechanisms may be involved in signal transduction by α_1-adrenergic receptors in some tissues.

This phenomenon has been most extensively studied in liver. Activation of α_1-adrenergic receptors in hepatocytes causes an increase in inositol phosphates which requires extracellular Ca^{2+} but an increase in cyclic AMP which is independent of extracellular Ca^{2+} (251). The effects of α_1-adrenergic agonists, vasopressin, and angiotensin II on metabolic responses in hepatocytes are all

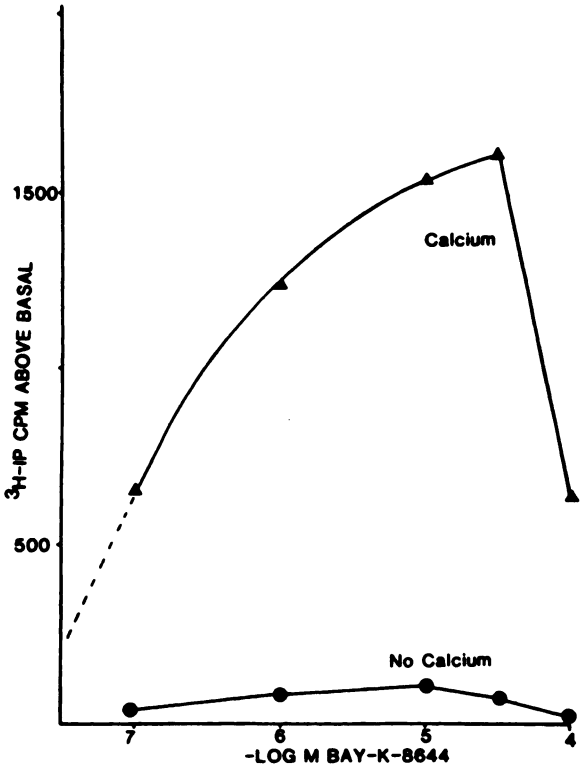


FIG. 2. Dose response relationship for the calcium channel agonist Bay K 8644 in increasing [^{3}H]inositol phosphate (^{3}H-IP) accumulation in slices of rat cerebral cortex in the presence and absence of calcium. Note that the activation of [^{3}H]inositol phosphate accumulation caused by this compound is dependent on the presence of Ca^{2+} in the extracellular medium. From Kendall and Nahorski (185) with permission.

thought to be due to changes in phosphatidylinositol metabolism and increasing cytosolic Ca^{2+} levels (214). However, the responses to α_1-adrenergic receptor activation persist in calcium-depleted hepatocytes where the effects of vasopressin and angiotensin are abolished (113, 187). Measurement of Ca^{2+} fluxes with ion-selective electrodes showed marked differences between α_1-adrenergic receptor stimulation and peptide receptor stimulation. The effects of α_1-adrenergic receptor stimulation were characterized by a net Ca^{2+} efflux, and no reuptake occurred until agonist was removed. Conversely, angiotensin and vasopressin caused a relatively small amount of Ca^{2+} efflux, a large Ca^{2+} influx, and a subsequent slower efflux (4). These results suggest that the mechanisms by which these receptors mobilize Ca^{2+} are substantially different. Thyroid deficiency reduces the effects of vasopressin and angiotensin II but not of α_1-adrenergic receptor agonists (68), while insulin reduces the effects of α_1-adrenergic receptor agonists but not of the peptides (79). Curiously, effects of α_1-adrenergic receptor agonists become completely dependent on external Ca^{2+} in livers from adrenalectomized rats (57, 113). Apparently corticosteroids are required for the Ca^{2+}-independent effects. Garcia-Sainz and Hernandez-Sotomayor (113) proposed that there are two mechanisms for the metabolic effects of α_1-adrenergic receptor activation in liver: one independent of external Ca^{2+}, inhibited by insulin, and modulated by glucocorticoids (presumably related to the cyclic AMP response); the other dependent on external Ca^{2+}, insensitive to insulin, and modulated by thyroid hormones (presumably related to the inositol phosphate response).

Similarly, in slices of rat brain, α_1-adrenergic receptor activation also increases the accumulation of both inositol phosphates (see above) and cyclic AMP (76, 168, 302, 307). As in liver, the α_1-adrenergic receptor-stimulated increases in inositol phosphate accumulation are blocked by chelation of extracellular Ca^{2+}, while α_1-adrenergic receptor-stimulated increases in basal cyclic AMP accumulation are unaffected by this procedure (169). In brain, however, another unusual phenomenon is observed. Although the increases in basal cyclic AMP accumulation caused by α_1-adrenergic receptor stimulation are relatively small, stimulation of this receptor type can greatly potentiate the increases in cyclic AMP accumulation caused by activation of other receptors. Similar potentiative effects can be observed between histamine H_1 receptors and other receptor types. For example, norepinephrine causes a much greater increase in cyclic AMP levels in slices of rat cerebral cortex than does either the specific β-adrenergic receptor agonist isoproterenol or the specific α_1-adrenergic receptor agonist 6-fluoronorepinephrine (73). Addition of 6-fluoronorepinephrine in the presence of isoproterenol causes a much greater than additive increase in cyclic AMP levels. This interaction between α_1- and β-adrenergic receptors has been well studied in many different brain regions (74, 171, 201) and appears to be greatest in the olfactory bulb (325). Similar potentiative interactions of α_1-adrenergic receptor activation are observed with adenosine (168, 170, 171, 302) and vasoactive intestinal peptide (VIP) (218, 219) receptor activation.

The mechanism by which α_1-adrenergic receptor activation potentiates cyclic AMP responses to activation of other receptor types in brain is still unclear. The potentiative interactions are generally lost when the tissue is homogenized and/or membranes are prepared. This suggests that the potentiative response does not involve direct interactions between receptors and/or G proteins, but may require involvement of some diffusible second messenger substances. If brain tissue is homogenized in an isotonic physiological salt solution (Krebs-Ringer bicarbonate buffer), potentiative interactions can still be observed although their magnitude is reduced (59, 72, 150). It has been shown that this homogenization procedure results in resealed "sac-like" entities which maintain the metabolic integrity and second messenger interactions of intact cells (72). Several hypotheses have been proposed to explain these potentiative interactions. Receptor-mediated increases in intracellular Ca^{2+} (310), release of adenosine (74), activation of protein kinase C (148), and activation of phospholipase A_2 (98, 268, 304) have all been proposed. However, the data are still con-

troversial (75, 169), and the complexity of brain slices makes it difficult to identify the exact events involved in this interaction. It is interesting, however, that adrenalectomy increases the α_1-adrenergic potentiation of cyclic AMP accumulation in brain slices (249, 250), while stress or administration of ACTH decreases this response (99, 183, 326). Thus this response in the brain is like Ca^{2+}-independent responses in the liver in that it is modulated by corticosteroids, although in opposite directions.

Similar α_1-mediated potentiation of cyclic AMP responses to activation of other receptor types is observed in rat pineal, which lends itself better to examination of the molecular mechanisms underlying this phenomenon. Klein et al. (186) showed that activation of α_1-adrenergic receptors greatly increased the magnitude of the induction of pineal enzyme activity normally observed following activation of β-adrenergic receptors. Subsequently, this group showed that, similar to previous work in brain slices, activation of α_1-adrenergic receptors potentiated the increase in cyclic AMP accumulation caused by β-adrenergic receptor activation in the pineal by more than 10-fold (349). Similar results were obtained when cyclic GMP accumulation was studied (349). These effects may be related to inositol phospholipid hydrolysis and activation of protein kinase C, since phorbol esters were found to mimic both the potentiative effects on enzyme induction (371) and cyclic AMP accumulation (333). However, these potentiative effects appear to require influx of Ca^{2+} from the extracellular fluid (331). Although α_1-adrenergic receptor activation clearly increases intracellular Ca^{2+} in these cells, this increase is sustained rather than transient and appears to be totally dependent on influx (332). This is inconsistent with a primary effect of Ins(1,4,5)P_3 on mobilization of intracellular Ca^{2+} in these cells. In fact, although inositol phosphates are formed following α_1-adrenergic receptor activation (133), these seem to be mainly Ins(1,4)P_2 and Ins(1)P; little Ins(1,4,5)P_3 can be observed (332, 370). This observation might be explained if phospholipase C was only transiently stimulated following receptor activation, as is observed with substance P in rat parotid acinar cells (234a). In this case, a rapid desensitization to substance P occurs, yet the Ins(1,4,5)P_3 released is sufficient to release intracellular Ca^{2+}. However, a continued buildup of inositol mono- and bis-phosphates over time during receptor activation argues against such a rapid desensitization.

One possible link between α_1-adrenergic receptor activation and increases in cyclic AMP accumulation is arachidonic acid. Partington et al. (268) reported that inhibitors of cyclooxygenase, such as aspirin and indomethacin, partially blocked the effects of α_1-adrenergic receptor activation on cyclic AMP accumulation in brain slices. Similar results have been reported by Schaad et al. (304), although others (98, 169) found no effects of these compounds on this system. More directly, it has been shown that α_1-adrenergic receptor stimulation activates phospholipase A_2 as well as phospholipase C activity in several cell types. In clonal cell lines from thyroid (46) and kidney (318) and in primary cultures of rat pineal (142), α_1-adrenergic receptor stimulation increases release of arachidonic acid. Burch et al. (46) showed that α_1-adrenergic receptor-mediated activation of phospholipases A_2 and C can be differentiated by pertussis toxin pretreatment (fig. 3), neomycin, and reducing extracellular Ca^{2+}. These authors suggested that different G proteins might be involved in the receptor-mediated activation of these two different phospholipases. Similar results were obtained by Slivka and Insel (318). Ho and Klein (142) presented evidence that increased cytosolic Ca^{2+} and activation of protein kinase C may be involved in α_1-adrenergic receptor-mediated release of arachidonic acid in pinealocytes. Therefore some responses to α_1-adrenergic receptor activation may be caused by arachidonic acid release, possibly via a different mechanism than stimulation of inositol phosphate formation. Conversely, stimulation of arachidonic acid release may be secondary to activation of other signal transduction mechanisms, such as influx of Ca^{2+} or activation of protein kinase C.

There may also be multiple signal transduction mechanisms for α_1-adrenergic receptor activation in the heart. Stimulation of these receptors increases inositol phosphate formation in cardiomyocytes (43), but also activates cyclic AMP degradation (47) apparently by activating a cyclic nucleotide phosphodiesterase. α_1-Adrenergic receptor activation also increases cell hypertrophy and spontaneous contractile activity in cultured cardiomyocytes (315). The hypertrophy and increased spontaneous activity can be induced independently under different experimental conditions (315). Steinberg et al. (324) showed that α_1-adrenergic receptor stimulation caused both positive and negative effects on the rate of contraction of cardiomyocytes cocultured with neurons,

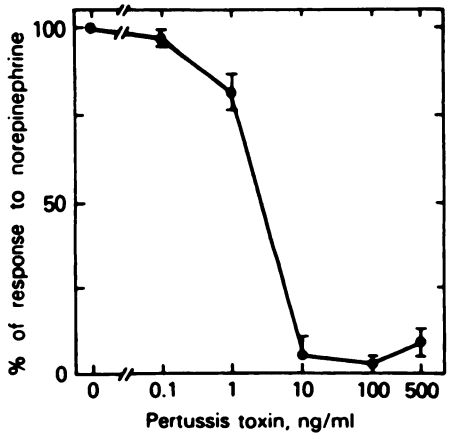


FIG. 3. Inhibition of α_1-adrenergic receptor-stimulated arachidonic acid release by pertussis toxin pretreatment of FRTL5 thyroid cells. This treatment had no effect on receptor-stimulated [^{3}H]inositol phosphate accumulation in these cells, suggesting involvement of a different G protein in these two effects. From Burch et al. (45) with permission.

and that only the negative chronotropic effect was blocked by treatment with pertussis toxin. It should be noted that pertussis toxin pretreatment does not block α_1-adrenergic receptor-mediated increases in inositol phosphates (43) or cyclic AMP degradation (47) in cardiomyocytes, or the positive chronotropic response in strips of rat ventricle (31).

Clearly, activation of α_1-adrenergic receptors has many diverse effects on cellular metabolism in addition to activating PIP_2 breakdown. These include activation of calcium influx, increasing cyclic AMP and cyclic GMP levels and responsiveness to other hormones, activating arachidonic acid release, increasing cyclic AMP degradation, and complex effects on cell growth. Many of these effects may occur secondary to the consequences of PIP_2 hydrolysis [release of $Ins(1,4,5)P_3$, phosphorylation to $Ins(1,3,4,5)P_4$, release of stored intracellular Ca^{2+}, and/or activation of protein kinase C]. However, increasing evidence suggests that some of these alternate effects are unrelated to receptor-mediated PIP_2 hydrolysis and may represent distinct signal transduction mechanisms. The possible relationship of these events to α_1-adrenergic receptor subtypes is discussed below.

VII. Origin of Increased Cytosolic Ca^{2+}

It is clear that many, if not all, of the cellular effects of α_1-adrenergic receptor activation are caused by increases in free ionized intracellular Ca^{2+}. Such increases could be caused by release from intracellular organelles and/or influx from the extracellular fluid. The recent advances in our understanding of inositol phospholipid metabolism and the role of $Ins(1,4,5)P_3$ in releasing Ca^{2+} from the endoplasmic or sarcoplasmic reticulum have focused attention on pools of intracellular Ca^{2+} (280). However, in many cases α_1-adrenergic receptor-mediated increases in cytosolic Ca^{2+} are partly or wholely dependent on influx from the extracellular fluid. The mechanism(s) by which receptor activation is linked to Ca^{2+} influx, and its relationship to inositol phospholipid metabolism, is currently receiving much attention.

A. Intracellular Ca^{2+}

There are several pools of stored intracellular Ca^{2+} which might be released following receptor activation. The three major subcellular regions in which Ca^{2+} is stored in most cells are the endoplasmic or sarcoplasmic reticulum, the mitochondria, and the inner plasma membrane (77, 285). The endoplasmic and sarcoplasmic reticulum and the mitochondria actively sequester Ca^{2+} with energy-requiring pumps, while the plasma membrane appears to contain molecules with a high affinity for binding Ca^{2+}, although these have not yet been identified. The endoplasmic or sarcoplasmic reticulum serves as the major source of activator Ca^{2+} in many cells (35, 192, 285). It is this pool which appears to be released by the $Ins(1,4,5)P_3$ released following receptor activation of PIP_2 hydrolysis (174, 329). The mitochondrial pool appears to function to stabilize the concentration of free cytosolic Ca^{2+}, by sequestering it when free Ca^{2+} is high and leaking it out when free Ca^{2+} is low (285). There is some evidence from liver that this pool is also regulated by receptor activation (29, 175), although this is controversial (285). The identity and function of Ca^{2+} bound to the inner plasma membrane are less clear. There is some evidence that this pool can be released by receptor activation (85), and it has been suggested that release of this small pool might trigger Ca^{2+} release from the sarcoplasmic reticulum (203).

B. Extracellular Ca^{2+}

There are also many different types of channels in the plasma membrane through which extracellular Ca^{2+} might enter the cell. These channels can be subdivided on the basis of their selective permeability to particular ions such as Ca^{2+}, and also by the stimulus which primarily controls channel permeability (34, 41, 234). Thus there are channels which open in response to changes in (*a*) trans-membrane voltage (voltage-operated channels, VOCs), (*b*) ligand occupation of a binding site on the channel complex (receptor-operated channels, ROCs), (*c*) increases in the concentration of a second messenger such as cyclic nucleotides or inositol phosphates inside the cell (second messenger-operated channels, SMOCs), and more recently (*d*) activation of G proteins by receptors or exogenous compounds (G protein-operated channels, GOCs). These classes undoubtedly have some overlap. For example, the permeability of VOCs can also be altered by changes in second messenger concentration (234).

Within each class of channels which are permeable to a particular ion and opened by a particular stimulus, there are also subclasses. Dihydropyridine-type Ca^{2+} entry blockers inhibit the slow inward Ca^{2+} current activated by depolarization of myocardial cells and other tissues (106, 162). However, they usually have little or no effect on depolarization-evoked transmitter release caused by opening of voltage-operated Ca^{2+} channels in presynaptic nerve terminals (108, 109, 125, 256, 312). Voltage-clamp and single channel conductance measurements have suggested the existence of multiple types of Ca^{2+} conductances (15, 111, 213, 330). Recently, three distinct types of Ca^{2+} channels have been identified in several cell types. These channels can be distinguished on the basis of the strength of depolarization required for channel activation, the time course of inactivation, and the sensitivity to dihydropyridine-type Ca^{2+} channel agonists and antagonists. "L type" channels require relatively strong depolarizations for activation, inactivate slowly, are at least partially blocked by dihydropyridine-type Ca^{2+} entry blockers, and potentiated by Ca^{2+} channel agonists. "T type" channels are activated by a smaller depolarization, inactivate rapidly, and are insensitive to dihydropyridines. "N type" channels also require a fairly strong depolarization for activation, inactivate with an

intermediate time course, and are also insensitive to dihydropyridines (108, 109, 263). These channels can also be distinguished by their sensitivities to blockade by cadmium (N and L more sensitive than T), nickel (T more sensitive than N or L), and omega-conotoxin (N and L more sensitive than T) (108, 223). Undoubtedly, more subtypes will be described as single channel recording techniques are increasingly applied to this area.

In addition to these voltage-operated Ca^{2+} channels, it has been suggested for some time that there may be receptor-operated Ca^{2+} channels (34, 351). The impetus for this suggestion came from observations that contraction of smooth muscle could be elicited by transmitter-receptor interactions even in muscles which were already depolarized with high potassium solutions (34, 100, 321). Also, norepinephrine can still produce changes in membrane permeability in potassium-depolarized smooth muscle (163, 217). It was also shown that contraction of some smooth muscles can be caused by transmitters and hormones in concentrations which do not appear to cause membrane depolarization (97, 135). Thus there has long been a major distinction between "electromechanical coupling" (contraction elicited by changes in membrane potential) and "pharmacomechanical coupling" (contraction elicited by receptor stimulation). Almost by definition, of course, electromechanical coupling is probably initiated by activation of VOCs. However, the mechanisms underlying pharmacomechanical coupling are undoubtedly more complex. Either ROCs, SMOCs, GOCs, or any combination could be involved, since all of these are activated by receptor occupation. Of course, the possible secondary involvement of VOCs opening in response to changes in membrane potential caused by changes in the permeability of the other channels, cannot be discounted. Clearly, the potential for confusion is great, and much more work will be necessary to clarify the situation.

There has been only one report of single channel recordings of a probable receptor-operated Ca^{2+} channel. Benham and Tsien (19) recently described a novel type of channel opened by ATP in arterial smooth muscle cells. These channels are 3-fold more permeable to Ca^{2+} than to Na^+ and are resistant to inhibition by cadmium, magnesium, and nifedipine. Since these channels were observed in excised patches, it was proposed that they are activated directly by ligand without the involvement of second messenger substances (19).

Also, very few Ca^{2+} channels activated by either second messengers or G proteins have been unequivocally identified and characterized. Kuno et al. (194) and von Tscharner et al. (361) have reported evidence for voltage-insensitive Ca^{2+} channels in T-lymphocytes and neutrophils which are activated by agonists. Kuno and Gardner (193) reported that the channels in lymphocytes were activated by $Ins(1,4,5)P_3$, supporting the possibility of a true SMOC. However, it is not always easy to distinguish between GOCs and SMOCs since receptor-mediated increases in second messenger accumulation usually also involve G proteins. For example, opioid peptides reduce Ca^{2+} currents in clonal neuroblastoma × glioma cells. This effect is blocked by pertussis toxin and restored by microinjection of certain G proteins (136). Similarly somatostatin, γ-aminobutyric acid (GABA), and norepinephrine have been reported to reduce Ca^{2+} currents in certain cells through mechanisms which are mimicked by GTP analogs and blocked by guanosine 5′-[β-thio] diphosphate (GDPβS) (92, 149, 206). However, Ca^{2+} currents are also reduced by activators of protein kinase C, such as synthetic diacylglycerols and phorbol esters (149, 206, 283). Yatani et al. (369) have presented evidence that G proteins directly affect Ca^{2+} channel survival in excised patches of guinea pig or bovine cardiac ventricular membranes, suggesting that, at least in some cases, there are direct effects of G proteins on Ca^{2+} channels without involvement of second messenger substances (369). Hopefully, as recording techniques get increasingly more sophisticated, more such channels will be identified and their properties delineated. At the moment, it is clear that there are multiple types of Ca^{2+} channels in cells, VOCs, ROCs, SMOCs, and GOCs, but the number and identities of the different types are not yet firmly established.

The question of which Ca^{2+} channels are blocked by organic Ca^{2+} entry blockers is still unclear. It was originally found that verapamil blocked potassium-evoked contractions much more effectively than norepinephrine-evoked contractions in smooth muscle (118, 125, 272). It was suggested by Bolton (34) that organic Ca^{2+} entry blockers blocked VOCs more readily than ROCs. Further analysis of many muscles revealed that, although depolarization-induced contractions were almost always very sensitive to inhibition by organic Ca^{2+} entry blockers, agonist-induced responses showed great variations in sensitivity (54, 162). In some tissues, norepinephrine-induced contractions were much less sensitive to inhibition by Ca^{2+} entry blockers than were depolarization-induced contractions (233, 308), while in other tissues the situation was reversed (26, 54, 364). From the single channel recording data discussed above, it is clear that some, but not all, voltage-operated Ca^{2+} channels are blocked by organic Ca^{2+} entry blockers. It is not yet clear whether any ROCs are blocked by these drugs. It is possible that the receptor-mediated responses blocked by organic Ca^{2+} entry blockers are secondarily mediated by VOCs, although the dissociation from changes in membrane potential (97, 135) makes this less likely. The situation is complicated further by the fact that the blockade of VOCs by dihydropyridine-type Ca^{2+} entry blockers is strongly state dependent, occurring most potently and effectively during prolonged depolarizations which open and/or inactivate the channels (108, 273, 284). Thus comparison of the potencies of these drugs at

various channels may be fraught with potential difficulties.

C. Reciprocal Effects

Of course the various sources of intracellular and extracellular Ca^{2+} do not exist in isolation from each other. The intracellular storage pools from which Ca^{2+} is released are rapidly refilled, either by Ca^{2+} leakage across the membrane, exchange for other ions, or influx through membrane channels. In many cells, it appears that there is an initial transient increase in intracellular Ca^{2+} in response to receptor activation which is caused by release from intracellular pools. Following this transient internally derived increase in cytosolic Ca^{2+}, however, there is a sustained influx of Ca^{2+} across the plasma membrane. This sustained influx probably serves to continuously activate the cell after intracellular stores are depleted and also to replenish the intracellular storage pools (278). It is likely that the initial release of stored intracellular Ca^{2+} may, in many cases, promote the secondary influx of extracellular Ca^{2+}. Kuno and Gardner (193) showed that $Ins(1,4,5)P_3$ might gate Ca^{2+} channels (see above). Putney (279) has recently suggested that a certain fraction of the endoplasmic or sarcoplasmic reticulum in cells which lies closely adjacent to the plasma membrane may have some direct connection which would allow Ca^{2+} entry directly from the extracellular fluid (possibly similar to a gap junction regulated by Ca^{2+} or second messengers). $Ins(1,4,5)P_3$-induced release of Ca^{2+} from this structure would then stimulate refilling by direct Ca^{2+} influx. Evidence for Ca^{2+}-activated Ca^{2+} channels has been reported (361). Alternatively, initial influx of Ca^{2+} caused by channel opening may promote release of stored Ca^{2+} (203, 278). Such "Ca^{2+}-induced Ca^{2+} release" has been proposed to play a major role in activation of smooth muscle contraction (177). Finally, it is likely that increases in cytosolic Ca^{2+} also speed inactivation of certain types of Ca^{2+} channels (179). The linkages between these phenomena are obviously complex and only beginning to be understood.

VIII. Role of Intra- and Extracellular Ca^{2+} in α-Adrenergic Receptor-Mediated Contractions of Smooth Muscle

Variabilities in the importance of extracellular Ca^{2+} in α-adrenergic receptor-mediated contractile responses are extremely intriguing and have received much attention in smooth muscle. It is clear that α_1- and α_2-adrenergic receptors coexist on many postjunctional smooth muscle cells, and that activation of either subtype activates contraction (90, 96, 198, 199, 227, 322, 339, 344, 345). The contractile responses caused by activation of either subtype are thought to be due to an increase in free cytosolic Ca^{2+} in the muscle cells (341). Since Ca^{2+} ions play an obligatory role in transferring the signal of receptor activation to contraction of myofilaments, changes in the contractile state of the muscle are often used as a bioassay for changes in the concentration of intracellular free Ca^{2+}. These increases in cytosolic Ca^{2+} cause, through calmodulin, activation of myosin light chain kinase. This enzyme phosphorylates the myosin light chain and thereby increases the interaction between actin and myosin. This results in contraction of the muscle cell (34, 352, 354).

In order to determine the importance of Ca^{2+} influx to a contractile response, one can simply remove extracellular Ca^{2+} or block influx with organic, (e.g., dihydropyridine) or inorganic (e.g., lanthanum, cobalt, cadmium, nickel) Ca^{2+} entry blockers. The contribution of stored intracellular Ca^{2+} can sometimes be determined by depleting releasable intracellular pools by repeated exposure to agonists or to caffeine and determining the residual contractile response. Coupling these two approaches allows one to determine (subject to equilibration between pools and limitations of interpretation) the relative contribution of intracellular Ca^{2+} mobilization and extracellular Ca^{2+} influx to an observed contractile response (33, 352).

As discussed above, it has been known for many years that α-adrenergic receptor-mediated contractions of different smooth muscles show a marked variation in the importance of extracellular Ca^{2+}. This is in contrast to depolarization-evoked contractions, which are essentially completely dependent on the presence of extracellular Ca^{2+} in every smooth muscle examined (33, 152, 153, 320, 353). The importance of extracellular Ca^{2+} for α-adrenergic receptor-mediated contractions depends on the particular muscle and animal species being used and the phase of contraction studied (36, 115, 353). Devine et al. (87) showed that there was a general correlation among different smooth muscles between the importance of extracellular Ca^{2+} in receptor-mediated contractile responses and the relative volume of sarcoplasmic reticulum in the muscle. For example, rabbit mesenteric vein had only 2.2% sarcoplasmic reticulum and depended completely on extracellular Ca^{2+} for agonist-induced contractions, while strips of main pulmonary artery had 5.1% sarcoplasmic reticulum and retained a significant contractile response to agonists in the absence of extracellular Ca^{2+} (87). The extensive ramifications of sarcoplasmic reticulum in skeletal muscle, where contraction is completely independent of extracellular Ca^{2+}, support such an interpretation. Possibly the size of the storage pool for releasable calcium influences the relative contribution which extracellular and intracellular Ca^{2+} make to agonist-mediated responses in smooth muscle, although this does not necessarily explain how receptor activation is linked to both sources. One possibility mentioned above and proposed by Putney (279) is that $Ins(1,4,5)P_3$ controls both the release of intracellularly stored Ca^{2+} and Ca^{2+} influx via the $Ins(1,4,5)P_3$-sensitive Ca^{2+} store.

A. Phasic versus Tonic

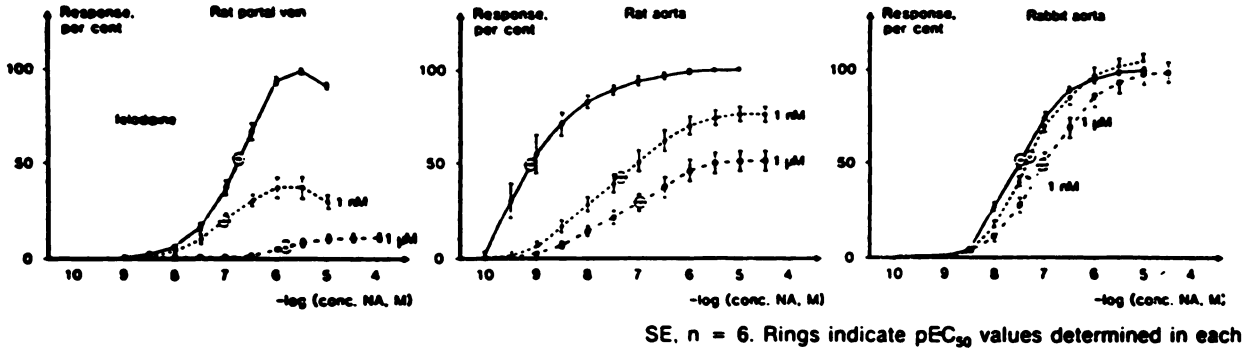
Bohr (32) suggested that contraction of rat aorta caused by epinephrine could be divided into two components. The initial fast phasic response (phase I) could be distinguished from a later slow tonic response (phase II). Similar two-phase responses can be observed in many other smooth muscles, and in some cases further components of contractions have been suggested (34). The relative sizes of these phasic and tonic components of contraction vary with the muscle studied and the contractile stimulus. In many muscles, the different phases of contraction have a different dependence on extracellular Ca^{2+} (27). Differential effects on different phases of contraction can complicate interpretations of studies on the importance of extracellular Ca^{2+} in smooth muscle contractile responses. However, there is no clear correlation between phasic and tonic contractions and the importance of Ca^{2+} influx. In rabbit aorta and ear artery, rapid phasic contractions caused by norepinephrine are not dependent on the presence of extracellular Ca^{2+} (86, 176), while in rat mesenteric arteries or resistance vessels, norepinephrine-induced rapid phasic contractions are abolished in Ca^{2+}-free medium (116, 264). In rat anococcygeus muscle, slow tonic contractions to norepinephrine are less sensitive to inhibition by Ca^{2+} entry blockers than are phasic contractions (227, 266), while the reverse holds true in rabbit aorta (85, 86). Clearly, differences in types of contraction cannot simply explain the differences in the importance of extracellular Ca^{2+} for smooth muscle contraction. This subject has recently been well summarized by Timmermans and Thoolen (341).

B. α_2- versus α_1-

Another possible explanation for the differential dependence on extracellular Ca^{2+} is that there might be different α-adrenergic receptor subtypes involved in contraction of different smooth muscles. van Meel et al. (355) first reported that a variety of organic Ca^{2+} entry blockers inhibited pressor responses elicited by the α_2-adrenergic receptor agonist 6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo[4,5d]azepine (BHT 920) in pithed rats without affecting pressor responses to the α_1-adrenergic receptor agonists phenylephrine or methoxamine. These authors also showed that manganese, nickel, and cobalt also selectively inhibited the response to α_2-adrenergic receptor agonists (356). Similar results were obtained by the same group (338, 357, 358), as well as others (56, 212) in other tissues. Similarly, the calcium entry promoter methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate (Bay K 8644) was shown to potentiate the pressor responses produced during α_2- but not α_1-adrenergic receptor activation (366). These data all supported the hypothesis that smooth muscle contraction mediated by α_2-adrenergic receptors required Ca^{2+} influx, while that mediated by α_1-adrenergic receptors did not. This hypothesis was consistent with the evolving mechanism of action of α_1-adrenergic receptor activation, since formation of Ins(1,4,5)P_3 and release of intracellular Ca^{2+} should not require influx of Ca^{2+} from the extracellular fluid.

However, further examination of the dependence of smooth muscle contraction on extracellular Ca^{2+} revealed several instances where α_1-adrenergic receptor-mediated contractions were clearly blocked by organic Ca^{2+} entry blockers. Timmermans et al. (340) showed that vasoconstriction in pithed rats caused by the selective α_1-adrenergic receptor agonist 2-(2-methyl-indazol-4-imino)imidazolidine (Sgd 101/75) was completely blocked by organic Ca^{2+} entry blockers. Further studies in pithed rats demonstrated that vasoconstrictor responses to some α_1-adrenergic receptor agonists are much more effectively blocked by Ca^{2+} entry blockers than are responses to other α_1-adrenergic receptor agonists (16, 342, 343). It has since become clear that α_1-adrenergic receptor-mediated contractile responses in many tissues both in vitro as well as in vivo are blocked by organic Ca^{2+} entry blockers, while in other tissues they are not affected (37, 67, 146, 208, 222, 230, 271, 290, 303). The generalization that all α_2-adrenergic receptor-mediated contractions of smooth muscle are potently blocked by organic Ca^{2+} entry blockers still holds true at the time this review is being written (341). However, α_1-adrenergic receptor-mediated contractions appear to be much more heterogeneous. Some such contractions are potently blocked by organic Ca^{2+} entry blockers, while others are not (341).

The differential effects of organic Ca^{2+} entry blockers on α_1-adrenergic receptor-mediated contractile responses in smooth muscle can be demonstrated in two different ways. First, contractile responses to the same agonist can be differentially affected in different muscles. For example, the contractile response to the selective α_1-adrenergic receptor agonist phenylephrine is markedly reduced by 0.01 μM nifedipine in dog circumflex coronary artery, but unaffected by 1 μM nifedipine in dog saphenous vein (255). Similarly, the contractile response to the selective α_1-adrenergic receptor agonist Sgd 101/75 is markedly reduced by 10 μM methoxy-verapamil-HCl (D600) in rat aorta, but not in guinea pig aorta or rat anococcygeus muscle (16, 359). Finally, 1 μM felodipine abolishes the contractile response to norepinephrine in rat portal vein, reduces it in rat aorta, but does not alter it in rabbit aorta (fig. 4) (211), even though α_1-adrenergic receptors seem to be mediating responses in all three tissues. In addition, contractile responses to different agonists in the same muscle can be differentially affected by organic Ca^{2+} entry blockers. In rat aorta, for example, contractile responses to the selective α_1-adrenergic receptor agonists 2-(2-chlor-5-trifluormethyl-phenyl-imino)imidazolidine nitrate (St 587) and 1,2,3,4-tetrahydro-8-methoxy(5-methylthio)-2-napthalenamine HCl



SE, n = 6. Rings indicate pEC_{50} values determined in each

FIG. 4. Effect of the organic Ca^{2+} entry blocker felodipine on contractile responses to norepinephrine in rat portal vein, rat aorta, and rabbit aorta. Note the markedly different sensitivities of each tissue to inhibition by felodipine. From Ljung and Kjellstedt (211) with permission.

(1-SK&F 89748-A) are profoundly reduced by 10 μM D600, while the contractile response to norepinephrine is altered very little (16, 222).

C. Role of Receptor Reserve

Several different hypotheses have been advanced to explain the differential sensitivities of α_1-adrenergic receptor-mediated contractile responses to blockade by organic Ca^{2+} entry blockers. It was suggested that differential receptor reserves could influence the degree to which α_1- and α_2-adrenergic receptor-mediated contractions could be blocked by these drugs (129, 293). Many of the agonists whose contractile responses were blocked by Ca^{2+} entry blockers had low intrinsic efficacies, while those whose contractile responses were not blocked had relatively high intrinsic efficacies. Responses to agonists with low intrinsic efficacies will always be easier to functionally antagonize than responses to agonists with high intrinsic efficacies, and such a difference could explain the differential susceptibility of responses to different agonists in the same tissue. A similar explanation could be invoked to explain the differential susceptibility of responses to the same agonist in different tissues, since the tissues could have different receptor reserves and therefore different "buffering" capacities (293).

Further work in this area has led to the general conclusion that variations in receptor reserves explains some, but not all, of the differences in the susceptibility of α_1-adrenergic receptor-mediated contractions to inhibition by organic Ca^{2+} entry blockers (166, 205, 260, 270, 337, 343). Differences remain even when the receptor reserves are eliminated.

D. Different Coupling States or Receptor Subtypes

Another explanation which has been suggested is that there may be distinct coupling states of the α_1-adrenergic receptor, and the receptor would activate different signal transduction mechanisms depending on the local tissue environment (230, 265). O'Brien et al. (265) examined the effects of manipulating arterial blood gases and inhibiting angiotensin converting enzyme on pressor responses to α-adrenergic receptor agonists in pithed rats. They concluded that, although these factors altered responsiveness to many agonists, they had no predictable relationship to the apparent receptor subtype involved. The possibility that a single receptor subtype initiates different signal transduction mechanisms depending on the local tissue environment will obviously be difficult to distinguish from the existence of two similar but slightly different receptors whose relative contributions to the response are dependent on the tissue environment (see below).

The simplest explanation which would account for the differential importance of Ca^{2+} influx in α_1-adrenergic receptor-mediated contractile responses would be the existence of two distinct receptor subtypes linked to different signal transduction mechanisms. Some evidence for this was obtained shortly after the phenomenon was first identified. McGrath (228) showed that the order of potencies of agonists in activating Ca^{2+} influx-dependent contractions of rat anococcygeus muscle was different from their order of potencies in activating responses independently of Ca^{2+} influx. Based on differences in the importance of Ca^{2+} influx in contractile responses to methoxamine and clonidine in rabbit aorta, Holck et al. (147) proposed that there might be different recognition sites for α_1-adrenergic receptor agonists, "with selective stimulation of the imidazoline site leading to transmembrane influx of extracellular Ca^{2+}." The existence of two different subtypes of α_1-adrenergic receptors linked to Ca^{2+} influx-dependent and -independent contractile responses was not supported by other studies. Korstanje et al. (191) and Beckeringh et al. (16) showed that several competitive antagonists had similar potencies in blocking both Ca^{2+} influx-dependent and -independent responses to α_1-adrenergic receptor agonists. On the other hand, it is always difficult to disprove the existence of pharmacologically distinct receptor subtypes, since other drugs not yet tested or newly discovered might better distinguish between closely related receptor subtypes. In the past few years, much evidence has accumulated that α_1-adrenergic receptors are not pharmacologically ho-

mogeneous, and that at least two distinct receptor types can be identified with appropriate antagonists. Han et al. (130, 131) have recently presented evidence that two distinct α_1-adrenergic receptor subtypes activate responses in smooth muscle which are differentially sensitive to inhibition by organic Ca^{2+} entry blockers. This evidence is discussed below.

IX. Direct Measurement of Ca^{2+} Mobilization and Fluxes

Two major approaches have been utilized to obtain more direct information on receptor-mediated alterations in cellular Ca^{2+} concentrations. Measuring the accumulation, loss, or unidirectional flux of ^{45}Ca in an intact tissue can give information on changes in Ca^{2+} handling in response to acute perturbations such as receptor activation. Conversely, fluorescent dyes and ion-selective electrodes have been employed in attempts to measure directly the intracellular concentration of free ionized Ca^{2+} in intact tissues or in dispersed or cultured cells.

Many studies of ^{45}Ca fluxes have been performed in various smooth muscles. In general, both depolarization and α_1-adrenergic receptor activation have been found to stimulate influx of Ca^{2+} from the extracellular fluid, but that only α_1-adrenergic receptor activation also releases intracellularly bound Ca^{2+} (10, 53, 55, 117, 350, 352). It seems likely, however, that the mechanisms by which receptor activation and depolarization promote Ca^{2+} influx are different. Meisheri et al. (233) measured net and unidirectional Ca^{2+} flux and compared them to contractile responses in rabbit aorta to determine whether these two stimuli caused influx via the same or different mechanisms. The use of selective inhibitors and studies of the additivity of the responses to the two stimuli showed that receptor activation and depolarization probably activate separate pathways for Ca^{2+} influx in this tissue (233).

Caffeine is known to release Ca^{2+} from the sarcoplasmic reticulum in cardiac and skeletal muscle (101), and it appears to have similar effects in smooth muscle. Although the mechanism of action is still unknown, it appears to be independent of actions on either phospholipase C or cyclic nucleotide phosphodiesterase. In many tissues, caffeine and α_1-adrenergic receptor activation appear to release Ca^{2+} from the same intracellular storage pool, although some differences are observed (83, 84, 126, 159, 204). This limited pool of intracellular Ca^{2+} can be depleted by caffeine or agonist exposure, but is rapidly refilled when extracellular Ca^{2+} is present (52, 176). This has led to the suggestion that there might be a physical coupling between the peripheral sarcoplasmic reticulum and the surface membrane (52) (see also ref. 279). If refilling is blocked (by lanthanum), the releasable pool is rapidly depleted by either caffeine or receptor stimulation, and repetitive stimulation causes no further mobilization of Ca^{2+} (352).

Several studies have attempted to relate α_1-adrenergic receptor-mediated release of intracellular Ca^{2+}, Ca^{2+} influx, and phosphatidylinositol metabolism in smooth muscle. Campbell et al. (51) showed that the biphasic contraction of rabbit aorta caused by α_1-adrenergic receptor stimulation was associated with a rapid hydrolysis of PIP_2 and a slower increase in ^{32}P incorporation into phosphatidic acid. These authors proposed that the rapid phasic response, mediated by release of intracellular Ca^{2+} (32), was caused by formation of $Ins(1,4,5)P_3$, while the sustained influx of Ca^{2+} resulting in tonic contractions was due to increases in phosphatidic acid, although this hypothesis has fallen into disfavor. Jim et al. (164) compared the abilities of a series of α_1-adrenergic receptor agonists to increase Ca^{2+} uptake and release internal Ca^{2+} in canine saphenous vein. They found that α_1-adrenergic receptor activation utilizes both intracellular and extracellular Ca^{2+} for contractions in this tissue, and that the increase in Ca^{2+} influx caused by different agonists was directly proportional to the intrinsic activities of the agonists in causing contractions (164). However, no correlation was observed between the efficacies of agonists in causing release of intracellular Ca^{2+} and contraction of saphenous vein (165). This raises the possibility that release of intracellular Ca^{2+} is not the sole mechanism initiating responses in this tissue. Conversely, Chiu et al. (61) reported that activation of α_1-adrenergic receptors in rat aorta caused both release of intracellular Ca^{2+} and influx of extracellular Ca^{2+}. Different agonists had different efficacies in stimulating these two different responses. However, the efficacies of the agonists in causing contraction appeared to depend more heavily on their ability to release intracellular Ca^{2+} (61). However, agonists which rely almost exclusively on Ca^{2+} influx to cause contraction in rat aorta (Sgd 101/75) did not cause release of intracellular Ca^{2+} or increase inositol phospholipid turnover (60). These data suggest, therefore, that the influx of extracellular Ca^{2+} is not subsequent to inositol phospholipid turnover in this tissue (60).

These studies again raise the possibility that release of intracellular Ca^{2+} and influx of extracellular Ca^{2+} in response to α_1-adrenergic receptor activation in smooth muscle may be due to two different molecular mechanisms. The differences in agonist efficacies for these two different effects support the possibility that two distinct receptor subtypes are involved (see below).

The relationship between α_1-adrenergic receptor activation and increases in cytosolic Ca^{2+} has been examined in a number of isolated cell preparations using fluorescent indicators such as 2-{[bis(carboxymethyl)amino-5-methylphenoxy]methyl}-6′-methoxy-8-bis(carboxymethyl)aminoquinoline-tetrakis(acetoxymethyl)ester (quin-2) or 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)ethane-N,N,N′,N′-tetraacetic acid (fura-2). In isolated hepato-

cytes, there appears to be a quantitative relationship between receptor-mediated formation of Ins(1,4,5)P_3 and mobilization of intracellular Ca^{2+} (174, 214). However, α_1-adrenergic receptor activation also promotes Ca^{2+} influx in liver (30, 288), and some metabolic responses seem to be dependent on this influx (288). Transient responses to α_1-adrenergic receptor activation, including ion fluxes and changes in oxidation-reduction ratios, appeared to be obligatorily dependent on the mobilization of intracellular Ca^{2+} and were independent of extracellular Ca^{2+}. However, sustained responses appeared to require Ca^{2+} influx (287). Recently, data from measurement of cytosolic Ca^{2+} in single hepatocytes have suggested that α_1-adrenergic receptor activation may promote repetitive Ca^{2+} transients, varying in frequency, but not in shape or amplitude (367, 368). Possibly, the frequency of such Ca^{2+} transients may be important in responses to Ca^{2+} mobilizing agents. The relationship of such oscillations to inositol phospholipid metabolism remains to be clarified.

Similar comparison of α_1-adrenergic receptor occupancy and increases in intracellular Ca^{2+} levels have been made in smooth muscle cells in culture. In some cases activation of α_1-adrenergic receptors mobilized Ca^{2+} from intracellular sites and also increased influx of extracellular Ca^{2+} (289). In other cases the effect of receptor activation appeared to be mainly due to release of intracellular Ca^{2+} (5–7, 314). Interestingly, Ambler et al. (6) recently showed that, during the period of maximal Ca^{2+} mobilization in a smooth muscle cell line, there was no measurable increase in Ins(1,4,5)P_3 levels.

It is apparent that the relationship between mobilization of intracellular Ca^{2+}, influx of extracellular Ca^{2+}, and increases in inositol phospholipid metabolism is not yet clear. Although Ins(1,4,5)P_3 clearly functions to mobilize intracellular Ca^{2+} in some instances in many tissues, it is not clear whether this is the only mechanism by which intracellular Ca^{2+} is mobilized. Similarly, the relationship of this event to the influx of extracellular Ca^{2+} remains to be determined.

X. Subtypes of α_1-Adrenergic Receptors

Increasing evidence suggests that α_1-adrenergic receptors can be further subdivided into pharmacologically distinct subtypes. Although there is still controversy as to the specificities of drugs, the mechanisms of signal transduction, and the localization of the various subtypes which have been proposed, many investigators now agree that α_1-adrenergic receptors in different tissues do not have identical properties (2, 95, 105, 138, 139, 244, 253, 254). There is still much confusion, however, about the exact nature of the differences between receptors in different tissues and the selectivity of various drugs.

A. Distinction from and Potential Similarity to α_2-Adrenergic Receptors

The subclassification of α_1-adrenergic receptors has been made much more difficult by the fact that α_2-adrenergic receptors coexist with α_1-adrenergic receptors in smooth muscle and also cause contractile responses when activated. α_1- and α_2-adrenergic receptors, although pharmacologically different, of course share many similarities. They are both activated by the naturally occurring catecholamines epinephrine and norepinephrine with approximately equal potencies, and many α-adrenergic receptor blocking drugs, including phentolamine, have similar potencies in blocking both subtypes. Although many agonists and antagonists exist which are relatively selective for either α_1 or α_2-adrenergic receptors, such selectivity is never absolute. Selective antagonists routinely have 50- to 1000-fold differences in their affinities for binding to α_1- and α_2-adrenergic receptors (80). Although such differences in affinity are sufficient to clearly differentiate these receptors pharmacologically, they are usually insufficient to completely block the response to one subtype but not another, particularly when high concentrations of agonists are used which might surmount competitive blockade.

Selective agonists present even more problems. Agonists may be selective in two different ways, either having different affinities in binding to the receptors or having different efficacies in activating them. These two parameters vary independently, and an agonist which selectively activates only a single subtype may bind to both equally well. Conversely, an agonist which has a higher affinity in binding to one subtype than the other may, once it is bound, activate both subtypes equally well. Again, specificity is usually only relative and not absolute. An agonist which selectively activates only a single subtype may have a small efficacy at the other subtype. Such problems are greatly compounded by the existence of differential receptor reserves in different tissues and for different receptor types. For example, clonidine has a much greater efficacy for activating α_2-adrenergic receptors than for activating α_1-adrenergic receptors (table 1), but in a tissue with a large α_1-adrenergic receptor reserve the contractile response to clonidine may be mediated by activation of α_1-adrenergic receptors (147). Similarly, two of the most selective α_2-adrenergic receptor agonists, BHT 920 and 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK 14, 304), have sufficient efficacies at α_1-adrenergic receptors to activate α_1-adrenergic receptor-mediated contractions in tissues with a large receptor reserve, such as rat and guinea pig aortae (17). Clearly, if there are two types of α_1-, and one or more types of α_2-adrenergic receptors activating contraction of a smooth muscle, and all of the drugs available can interact with all of the receptor subtypes at appropriate concentrations, the potential for confusion is very great.

Experimentally, this means that all assumptions must be constantly checked and rechecked when using different drugs, or when using the same drug under different conditions or in different tissues. Values for log concentration of antagonist occupying half total receptor pop-

ulation (pA_2) must always be determined for antagonists, and the slopes and shapes of the Schild plots must be carefully scrutinized (8, 182). The types of receptors activated by agonists must be carefully verified under each experimental condition and with each new drug and drug concentration tested. The extent of the receptor reserve must also be taken into account (112, 182). For example, a particular agonist which may be causing contraction in control tissues through an α_1-adrenergic receptor may, after partial receptor inactivation or addition of an α_1-selective competitive antagonist, begin to cause contraction through α_2-adrenergic receptors. Unfortunately, because of practical limitations in the use of many different drugs and tissues, absolute control of these parameters is not always possible.

Because of this complexity, it is perhaps not surprising that many confusing and conflicting data have been generated. α_2-Adrenergic receptor-mediated pressor responses can be readily observed after α_1-adrenergic receptor blockade in pithed rats (200, 227, 344). However, it has been much more difficult to demonstrate similar responses in vitro. Only in a few isolated tissues, such as dog saphenous vein (81), rabbit saphenous vein (309), and dog cerebral arteries (300), can clear α_2-adrenergic receptor-mediated contractile responses be observed in vitro.

On the other hand, some isolated tissues appear to have α-adrenergic receptors mediating contractile responses with pharmacological characteristics which seem to be intermediate between the α_1- and α_2-adrenergic receptor subtypes. Ruffolo et al. (299) first showed that rat aorta was exceptionally sensitive to contraction caused by clonidine, and that this response was potently inhibited by yohimbine (297). Based on these results, these authors proposed that rat aorta had α_2-adrenergic receptors on the postsynaptic smooth muscle cells. Further work substantiated the differences between the α-adrenergic receptors mediating contraction of rat aorta and those of other species (298). However, the classification of this receptor as being of the α_2-subtype was clearly suspect, since both α_1-selective agonists (phenylephrine and methoxamine) and antagonists (prazosin) are quite potent in this tissue (91, 94, 294). Ruffolo et al. (298) concluded that the α-adrenergic receptors of rat aorta "possesses properties of both alpha-1 and alpha-2 adrenergic receptors" (fig. 5). Further analysis of these receptors, however, has shown that they must clearly be classified as α_1. The contractile effects of all agonists studied, including highly α_1-selective agonists such as phenylephrine and methoxamine and highly α_2-selective agonists such as BHT 920, are much more sensitive to blockade by prazosin than by yohimbine (16, 78, 216). Although selective α_2-adrenergic receptor antagonists such as yohimbine and rauwolscine are more potent in rat aorta than in blocking α_1-adrenergic receptor-mediated contractile responses in other tissues, the α_1-selective antagonist prazosin is also more potent in rat aorta than in blocking α_1-adrenergic receptor-mediated contractile responses in other tissues (88, 128) (see below). With both α_1- and α_2-selective agonists, prazosin is more than 300-fold more potent than yohimbine in blocking contractile responses of rat aorta, indicating that this receptor is clearly of the α_1-subtype. Why both prazosin and yohimbine, and other drugs such as clonidine, are unusually potent in this tissue is not yet clear.

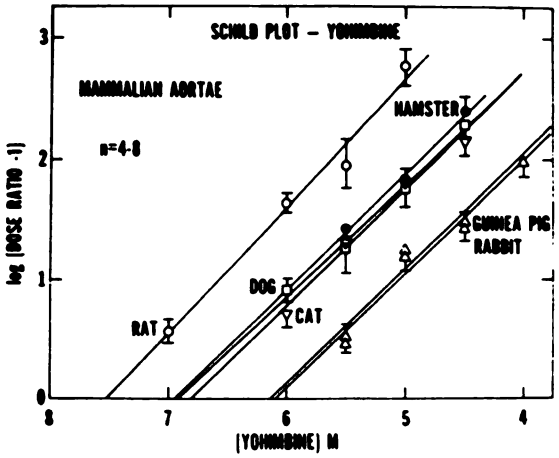


FIG. 5. Differences in the pA_2 values for yohimbine in blocking norepinephrine-stimulated contractions in aortae from 7 different species. The more than 30-fold differences in potencies observed in the different tissues suggest that the receptors have different pharmacological properties. From Ruffolo et al. (298) with permission *Bars*, SE.

There are also tissues where the difference between the affinities of prazosin and yohimbine is not so large (139). Prazosin is only 20-fold more potent than rauwolscine in blocking norepinephrine-induced contractions of cat mesenteric arteries (316), only 10-fold more potent in dog splenic artery, and only 3-fold more potent in dog splenic vein (141). This raises the possibility that both α_1- and α_2-adrenergic receptors might contribute to contractile responses in these muscles. However, if α_2-adrenergic receptors were contributing to the contractile response to norepinephrine in dog splenic vein, then α_2-selective agonists such as BHT 920 should also produce a response. But, BHT 920 does not activate contraction in dog splenic vein (141) although it is very effective in tissues with clear α_2-adrenergic receptor-mediated contractile responses, such as dog saphenous vein (107). The uniformly high affinity of these receptors for prazosin suggests that each of these receptors is of the α_1-subtype. However, the variable affinity for yohimbine and rauwolscine raises the possibility that there are distinct subtypes of this receptor with differing affinities for α_2-selective antagonists. Conversely, the pharmacological properties of the receptor may be influenced by its tissue environment, or some other unknown factor (see below).

B. Differentiation using Selective Agonists

In the studies described above, α_1-adrenergic receptors were differentiated by their affinity for drugs which are

usually α_2-selective. Many studies have also suggested that other drugs can also differentiate between α_1-adrenergic receptors in different tissues. Based on small differences in pA_2 values for phentolamine in different tissues, Sheys and Green (313) undertook a careful comparison of the pharmacological properties of the α-adrenergic receptors mediating contraction of rabbit aorta and spleen. They found relatively large differences (up to 10-fold) in the K_a values for agonists in activating contraction in the two tissues, with agonists being more potent in aorta. These data raised the possibility that the α-adrenergic receptors in rabbit aorta and spleen might differ from each other (313). Many other studies have subsequently substantiated the fact that the α-adrenergic receptors in rabbit aorta generally show a much higher affinity for norepinephrine (a lower functional K_a value) and other phenethylamine agonists than is found in other tissues (24, 181, 241, 242, 244, 281, 334) (see below).

1. Imidazolines versus phenethylamines. In examining desensitization of rat vas deferens, Ruffolo et al. (295) showed that long-term exposure to imidazoline-type agonists would decrease the contractile responsiveness to this class of agonists, but not to the phenethylamine class of agonists. This differential desensitization to agonists of different structural classes might be explained by the existence of two different types of α_1-adrenergic receptors, or by different attachment sites on the same receptor. These studies stimulated much interest in comparing these two classes of drugs, but are complicated by the fact that most imidazoline-type agonists have a much lower efficacy than phenethylamine-type agonists (80), and only maximal doses of drugs were tested. In this case, a marked reduction in receptor reserve might reduce the response to low efficacy agonists (imidazolines) much more than to high efficacy agonists (phenethylamines). Although Ruffolo et al. (295) argued against this explanation of their data, it was not clearly ruled out.

Based on a review of the literature and work from his own laboratory, McGrath (227) raised the possibility that α_1-adrenergic receptors should be divided into two subclasses, α_{1a}- and α_{1b}-. His arguments were based mainly on (*a*) the complexity of agonist dose-response curves, which sometimes consist of two components (25); (*b*) the differential effects of imidazolines and phenethylamines (295, 299); (*c*) the differences in types of contractile responses (226); and (*d*) differences in the potencies of antagonists in blocking agonist- and nerve-mediated contractile responses (224, 226).

Another imidazoline agonist has been used to obtain evidence for the existence of discrete subtypes of α_1-adrenergic receptors. Sgd 101/75 was shown to have markedly different intrinsic activities relative to norepinephrine in activating contraction of different smooth muscle preparations (157). Coates et al. (62) showed that low concentrations of the irreversible alkylating agent phenoxybenzamine preferentially reduced the effects of Sgd 101/75 relative to those of norepinephrine in rat anococcygeus muscle. When the effect of Sgd 101/75 was abolished by pretreatment with high concentrations of phenoxybenzamine, norepinephrine could still activate a contractile response in the continued presence of Sgd 101/75 (which should then be acting as a competitive antagonist). Similar results were obtained when benextramine was used as the alkylating agent (63). These results suggested that rat anococcygeus muscle contained two α_1-adrenergic receptor subtypes, both of which were activated by norepinephrine but only one of which was activated by Sgd 101/75. The receptor sensitive to Sgd 101/75 (α_{1a}) appeared to be preferentially inactivated by both phenoxybenzamine and benextramine (62, 63). However, further analysis of the receptors mediating contraction of rat anococcygeus muscle has not supported this hypothesis. Kenakin (181) carefully examined the effect of receptor alkylation on the responses to an imidazoline (oxymetazoline) and a phenethylamine (norepinephrine) in rat anococcygeus and vas deferens. He found that the differential responsiveness to these two drugs could be explained by different receptor reserves, and that a variety of selective antagonists showed no differences in affinity for the receptors (181). McGrath (229) obtained similar results with selective antagonists. James and Leighton (161) directly examined the effect of Sgd 101/75 on rat anococcygeus muscle and showed clearly that this drug was activating the same receptors as norepinephrine. When the response to Sgd 101/75 was abolished following partial receptor inactivation, this compound could competitively antagonize the response to norepinephrine with a potency similar to its potency in activating the receptors. James and Leighton (161) suggested that previous results had been confused by technical problems, such as insufficient time of drug equilibration with tissue. Although this was originally thought to be one of the first pharmacological distinctions between true α_1- (not α_2-like) adrenergic receptors in different tissues, it is now clear that the differences were related to the various technical problems involved in using agonists discussed above.

The studies suggesting heterogeneity between the α-adrenergic receptors causing contractile responses in aortae from different species discussed above led Ruffolo and Waddell (296) to determine the affinities and relative efficacies of a series of imidazoline agonists in rat and rabbit aortae. They found that the affinities of the drugs varied substantially between the two tissues. In fact, the rank order of affinities was exactly opposite for the receptors in rat and rabbit aortae. Similarly, large differences in the efficacies of these compounds relative to norepinephrine were observed in the two tissues (296). Clonidine was about 100-fold more potent in binding to the receptors in rat than rabbit aorta, although it had similar efficacies in both tissues. On the other hand,

oxymetazoline was only slightly more potent in binding to the receptors in rabbit than rat aorta, but had an almost 20-fold greater efficacy in rabbit aorta (296). Similar conclusions were reached by Digges and Summers (88), who compared contractile responses in rat aorta and rat portal vein. These authors found that the affinity of clonidine was approximately 20-fold higher in contracting aorta than portal vein, while the affinity for oxymetazoline was about 20-fold lower in contracting aorta than portal vein (88). However, the K_a values for both clonidine and oxymetazoline in rat aorta determined by Digges and Summers (1.3 and 15 μM, respectively) were about 20-fold higher than those found by Ruffolo and Waddell (296) in the same tissue (0.04 and 0.7 μM, respectively). The reason for this discrepancy is not clear, but could be related to animal or technical differences between laboratories. However, these studies support the hypothesis that the α_1-adrenergic receptors in different tissues have different pharmacological properties.

Similar results have been obtained in comparison of the properties of the α_1-adrenergic receptors mediating contraction of the ear artery and thoracic aorta of the rabbit (276, 277). Following previous reports that the affinities for agonists were substantially higher in rabbit aorta than in other tissues (see above), Purdy and Stupecky showed that the affinity of norepinephrine in activating contractile responses (K_a values) varied by more than 30-fold between rabbit ear artery (4 μM) and aorta (0.1 μM). K_a values for epinephrine varied by 200-fold, while K_a values for clonidine and methoxamine were not different between the tissues (276, 277).

2. Receptor microenvironment. When efficacies and K_a values for agonists are carefully determined following partial receptor inactivation, significant differences between these parameters in different tissues are generally thought to reflect the existence of pharmacologically distinct receptor subtypes. Recently, however, Bevan and colleagues have proposed that agonist affinity for a receptor is not an intrinsic property of the receptor molecule, but may vary depending on the tissue environment in which the receptor is found. Bevan et al. (28) determined 50% effective concentration (EC_{50}) and K_a values for norepinephrine in 12 rabbit arteries and found that there was more than a 200-fold difference between the K_a values determined in different arteries. Interestingly, the K_a values correlated strongly with the EC_{50} values determined before partial receptor inactivation, suggesting that the potency of norepinephrine is determined mainly by differences in affinity for the receptors in different tissues, rather than by differences in receptor reserve (fig. 6) (28). Less variation was found in the K_a values for phenylephrine (<20-fold). Some variation was found in the potency of the antagonist prazosin (10-fold), although this was much less than that found with norepinephrine (267). Such data could be interpreted as supporting the existence of two different receptor subtypes with different affinities for agonists, but existing in different proportions in different arteries. However, because of the impressive correlation between the observed EC_{50} and the calculated K_as in these arteries, Bevan et al. (28) proposed that the affinity of a receptor for an agonist might be a locally regulated characteristic and is not necessarily constant across different tissues, where receptors are in different microenvironments.

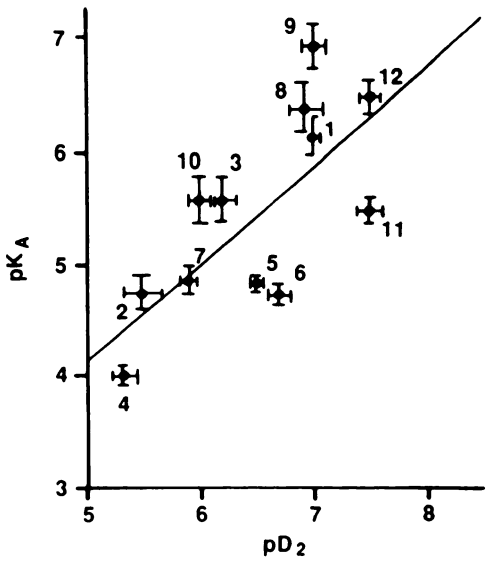


FIG. 6. Correlation between $-\log EC_{50}$ (pD_2) and $-\log K_a$ (pK_A) for norepinephrine-induced contractions in 12 different rabbit arteries. The strong correlation suggests that agonist affinity may be primarily responsible for differences in the sensitivity of different blood vessels to contraction by norepinephrine. From Bevan et al. (28) with permission.

These data are very intriguing, although their interpretation is far from clear. It has long been assumed by pharmacologists that the affinity of a drug for its receptor is a physical constant dependent only on the structure of the drug and the structure of the receptor binding site. The local tissue environment in which the receptor is found will, of course, affect the ability of the receptor to initiate a response. However, this factor has been thought to have little effect on the affinity of the receptor in binding drugs. Only covalent modifications of receptor structure would be expected to affect the kinetics of interactions between drugs and their receptor binding sites. This assumption has been well tested with antagonists. The binding constant for an antagonist has been generally shown to be the same for a particular receptor type, regardless of the tissue in which it is found (8, 182). In fact, differences in antagonist affinity constants are considered to be diagnostic of differences in receptor subtypes. Recently, with the application of biochemical techniques to the study of receptors, it has been shown that the affinity of a receptor for an antagonist is not altered even after solubilization of the receptor molecule from its native environment and purification to homgeneity (48).

The situation with agonists is much more complex. First, determination of agonist affinity constants in isolated tissues is much more difficult and dependent on assumptions about the specificity of alkylating agents and the relationship of occupancy to response (182) which have been subjected to little experimental verification. Therefore there is still uncertainty about the accuracy and molecular interpretation of agonist affinity constants determined in this manner. It is equally difficult and complicated to determine agonist affinity constants in direct radioligand binding assays. The affinities of agonists in radioligand binding experiments are commonly found to be dependent on the experimental conditions under which the binding is monitored. Much of this complexity is probably caused by the nature of agonist-receptor interactions, since the agonist must not only bind to the receptor, but must also induce a conformational change and activate a response. Many receptors, when occupied by agonist, associate with G proteins within the membrane (see above), and the association of the agonist-bound receptor with the G protein greatly reduces the dissociation rate of the agonist. GTP causes a dissociation of the complex and speeds up agonist dissociation kinetics (114). Thus the apparent affinity of agonists can be influenced by the presence of G proteins, guanine nucleotides, and other substances which can influence these interactions such as divalent cations.

α_1-Adrenergic receptors clearly exist in multiple affinity states in membrane preparations, and these affinity states are interconverted by nucleotides and cations (38, 66, 123, 160, 215, 311, 319, 335). It is possible that these different affinity states are differentially involved in contractile responses in different tissues. However, the differences between the high and low affinity binding states are usually less than 50-fold, substantially smaller than the differences in apparent K_a values observed in different arteries (28). It is also difficult to understand how the effect of receptor activation might be mediated by different receptor affinity states in different tissues. Our current understanding of the different affinity states is that they represent free receptor (rapid agonist dissociation, low affinity) or receptor complexed with a G protein (slow agonist dissociation, high affinity). Since the interaction between the receptor and the G protein is probably the initial event in signal transduction, and all activated receptors presumably have to complex with G proteins to activate their response, it is difficult to understand how this will differ in different tissues unless either the receptor or the G protein is different.

3. Comparison with binding studies. In fact, when agonist affinity constants have been compared using radioligand binding methods, there has been little evidence to support the differences in affinity between the α_1-adrenergic receptors in different tissues discussed above. Because of tissue limitations it is often difficult to perform binding studies on the smooth muscles in which contractile responses are examined. However, in large muscles like aorta, such studies can sometimes be successful, particularly if high specific activity probes are used to label the receptors. The extremely high affinity for norepinephrine, which has been observed in many functional studies on rabbit aorta (24, 276, 281, 313, 334), has not been confirmed with radioligand binding techniques. Two studies have reported direct labeling of α_1-adrenergic receptors with the high affinity radiolabeled antagonist ^{125}I-{2-[β-(4-hydroxyphenyl)ethylaminomethyl]tetralone} (^{125}IBE) (346, 365). Norepinephrine shows apparent K_d values of 4 μM and 11.5 μM in competing for these binding sites (346, 365). This reflects a substantially lower affinity than the K_a values which have been determined in functional studies. It is also much closer to the K_a values reported in rabbit ear artery (4 μM; 277) and many other tissues (244) than in rabbit aorta (0.1 μM; 277). The K_d value for norepinephrine in binding to α_1-adrenergic receptors in rabbit aorta is also not substantially different from the K_d values reported for norepinephrine from binding studies in many other tissues, taking into account differences in experimental conditions such as temperature, nucleotides, and cations (244). Similarly, differences in functional K_a values for epinephrine between rabbit aorta (0.03 μM) and ear artery (6 μM; 277) are also not observed in direct binding assays of membranes from rabbit aorta, where epinephrine had a K_d of 4 μM (346) or 9 μM (365), similar to that in other tissues (244).

It would be of great interest to determine the binding properties of imidazolines such as clonidine and oxymetazoline at the α_1-adrenergic receptor binding sites in different smooth muscles, particularly rat aorta. Direct binding studies on rat aorta have been reported (82, 173), although the affinities for imidazolines were not studied. In examining the properties of α_1-adrenergic receptors in rat brain labeled by ^{125}IBE, however, oxymetazoline appeared to behave differently than any of the other agonists or antagonists studied (239). Under the binding conditions used, all agonists and antagonists except oxymetazoline had Hill coefficients close to 1.0 for inhibition of specific ^{125}IBE binding to membrane preparations of rat cerebral cortex. Scatchard analysis of ^{125}IBE binding in the presence of different concentrations of competing drugs showed that, although other agonists and antagonists caused a purely competitive inhibition, the inhibition caused by oxymetazoline appeared to consist of two components (fig. 7), consistent with two different binding sites with different affinities for oxymetazoline (239). Similar results were obtained in binding studies on membranes from rat vas deferens (245). However, other imidazolines such as clonidine and tramazoline did not show such differences (239, 245). The relationship of these differences to the different K_a values found in different smooth muscles remains to be determined. However, these binding data are consistent with the two

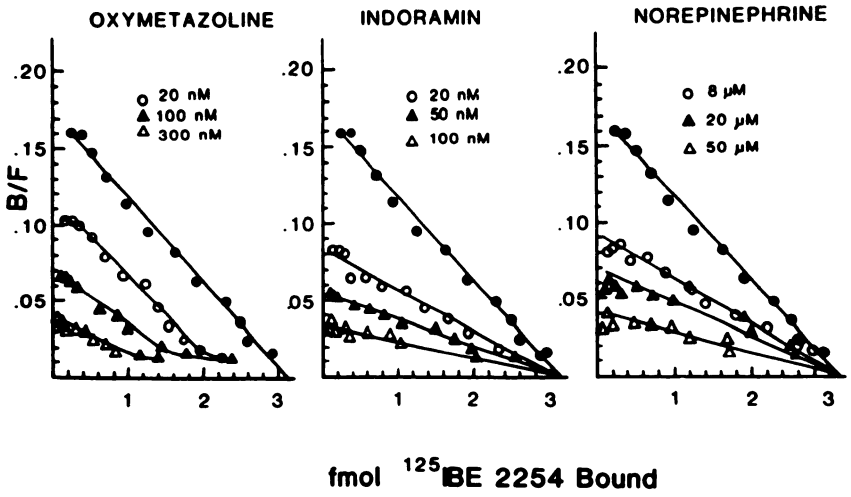


FIG. 7. Complex behavior of oxymetazoline in competing for specific ^{125}IBE binding sites in membranes from rat cerebral cortex. Note that the antagonist indoramin and the agonist norepinephrine show simple competitive behavior, while oxymetazoline distinguishes between two binding sites with different affinities. *B/F*, bound/free. From Minneman (239) with permission.

types of α_1-adrenergic receptor binding sites distinguished by the antagonist 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane HCl (WB 4101) discussed below.

Colucci et al. (66) raised the possibility that contraction of rabbit aorta might be mediated by the high agonist affinity binding state of the receptor (presumably complexed with a G protein). These authors showed that the binding of norepinephrine to membranes from cultured rabbit aorta smooth muscle cells was best described by a two-site model in which about half of the sites had a K_d of 0.1 μM and the other half had a K_d of 7 μM. The relationship between receptor occupancy (determined in membranes) and stimulation of ^{45}Ca efflux (determined in whole cells) was linear for the high affinity sites, but convex for the low affinity sites. However, these authors also showed that, following receptor alkylation by phenoxybenzamine, there was a large loss of receptor binding sites but little loss of receptor-stimulated ^{45}Ca efflux, indicating a receptor reserve for this phenomenon. In the presence of a receptor reserve, the occupancy-response relationships will be convex (182, 292). Thus the linear relationship between norepinephrine-stimulated Ca^{2+} efflux and occupation of the high affinity state of the receptor (66) may be fortuitous. On the other hand, there may be some mechanistic relationship we do not yet understand.

Differences in the potencies and efficacies of agonists in promoting α_1-adrenergic receptor-mediated accumulation of different second messenger substances have also been reported. In liver and brain, α_1-adrenergic receptor activation increases cyclic AMP accumulation and potentiates the cyclic AMP response to activation of other receptors, as well as increasing inositol phosphates (58, 76, 168, 302, 307). Morgan et al. (252) showed that there were some pharmacological differences between the α_1-adrenergic receptors controlling cyclic AMP accumulation and phosphorylase activation in rat liver. Similarly, Johnson and Minneman (168) showed pharmacological differences between the α_1-adrenergic receptors increasing inositol phosphate and cyclic AMP accumulation in slices of rat cerebral cortex. In particular, the synthetic phenethylamine agonists phenylephrine and methoxamine had substantially higher intrinsic activities in activating inositol phosphate accumulation (50 to 60% of norepinephrine) than in activating cyclic AMP accumulation (7 to 19% of norepinephrine). Only one imidazoline agonist was studied, St 587, which had no measurable intrinsic activity for stimulating either response (168).

In their totality, these data suggest that there are intriguing differences between the affinities and efficacies of agonists at α_1-adrenergic receptors in different tissues. Most such data have so far been obtained from functional studies, where both K_a and efficacy values are dependent on experiments performed after partial receptor inactivation with highly reactive and potentially nonspecific alkylating agents. The most likely explanations for the observed differences include (*a*) the existence of discrete receptor subtypes, (*b*) modulation of receptor affinity by tissue microenvironment, (*c*) participation of different affinity states of a single receptor in responses in different tissues, or (*d*) technical or theoretical problems in calculating K_a and efficacy values in functional experiments. So far, the small amount of data available from radioligand binding assays which addresses this problem does not support the differences in receptor affinity for agonists which have been reported in functional studies. However, further work is clearly needed in this area to distinguish between these possibilities and to resolve these potentially important pharmacological differences.

C. Differentiation using Selective Antagonists

Subclassification of receptors using selective antagonists is generally much less difficult to interpret than

subclassification using selective agonists. Since antagonists bind to the receptor but do not activate it, they are characterized only on the basis of their affinity constants. There is no complication with differences in efficacy. Also, the degree of receptor blockade is absolutely proportional to the degree of receptor occupancy by a competitive antagonist, and therefore the actual binding constant of the antagonist for the receptor under study can be determined relatively easily. If appropriate precautions are taken and certain criteria fulfilled, the pA_2 for an antagonist in blocking the response to an agonist should be an accurate indication of its affinity in binding to the receptor site (8, 182, 292).

When the pA_2 values for antagonists in blocking α_1-adrenergic receptor-mediated responses have been compared to their K_d values in competing for α_1-adrenergic receptor binding sites in membrane preparations from the same (151, 231, 243, 245, 247, 301, 305) or different (140, 239, 336) tissues, the correlation has been impressive (fig. 8). These data have supported the interpretations that (*a*) the affinity of α_1-adrenergic receptors for antagonists is not altered by homogenizing tissues and making membrane preparations; and (*b*) the pharmacological properties of α_1-adrenergic receptors are similar in most tissues.

However, apparent differences between pA_2 values for antagonists have been observed at α_1-adrenergic receptors in different tissues. As mentioned above, Sheys and Green (313) noted that the pA_2 for phentolamine differed by 2- to 3-fold in different tissues. More dramatic differences were observed when pA_2 values for yohimbine were compared in aortae from different species (297), although this was originally thought to be due to differential involvements of α_2-adrenergic receptors (see above). It

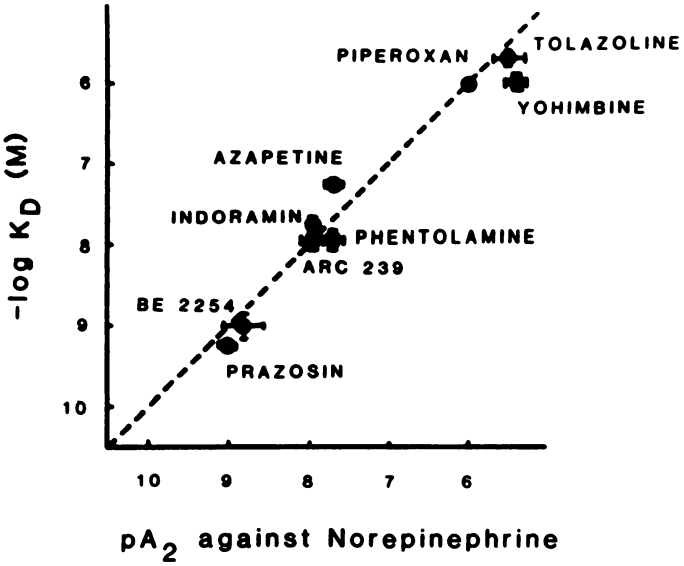


FIG. 8. Similarity of pA_2 and pK_d values for antagonists in rat vas deferens. These data suggest that the pharmacological properties of receptor binding sites in membranes do not differ from those in intact tissues, at least for antagonists. From Minneman et al. (245) with permission.

now seems likely that differences in affinity for α_2-selective antagonists could reveal differences in the pharmacological properties of α_1-adrenergic receptors in different tissues, although the potential for confounding effects on α_2-adrenergic receptors is always difficult to completely eliminate.

1. Prazosin. The use of α_1-selective antagonists is much more straightforward. Since these drugs will block α_1-adrenergic receptors in concentrations which can be clearly shown to have no effect on α_2-adrenergic receptors, differences in apparent affinity for receptors in different tissues can more easily be attributed to the existence of discrete subtypes of α_1-adrenergic receptors. Several reports have appeared, suggesting that the prototype α_1-adrenergic receptor antagonist prazosin might have different affinities for α_1-adrenergic receptors in different tissues. Digges and Summers (88) showed that prazosin was about 10-fold more potent in blocking norepinephrine-mediated contractions of rat aorta (pA_2 of 9.4) than rat portal vein (8.4). Interestingly, similar 10-fold differences were observed for most other antagonists tested {2-[β-(4-hydroxyphenyl)ethylaminomethyl]tetralone (BE 2254), phentolamine, and rauwolscine} except, surprisingly, for yohimbine. In studying contractions of rabbit main pulmonary artery, Holck et al. (147) found that prazosin competitively blocked contractions caused by both clonidine and methoxamine, but was significantly more potent in blocking responses to clonidine (pA_2 of 9.4) than methoxamine (8.4). In this case, similar differences were found for yohimbine (pA_2 of 6.6 against clonidine and 5.8 against methoxamine). Since clonidine is a relatively α_2-selective agonist while methoxamine is relatively α_1 selective, it was surprising that the α_1-selective antagonist prazosin was more potent against clonidine than methoxamine. Conversely, Medgett and Langer (232) reported that prazosin was about 10-fold more potent in blocking contractile responses of rat tail artery to methoxamine [log concentration of antagonist shifting agonist dose-response curve 2-fold (pK_B) of 9.7] than norepinephrine (8.9). In examining the literature, Medgett and Langer (232) and Agrawal et al. (2) noted that there was a very wide range in the pA_2 values reported for prazosin in different tissues. The values ranged from a low of 11.2 in rat aorta (78) to a high of 7.2 in rabbit renal artery (71). Although in some cases there may have been some noncompetitive actions of prazosin (282) or the involvement of α_2-adrenergic receptors, in general the variations appear to reflect true differences between the receptors in different tissues. On the other hand, much of the variation comes from a single tissue, rat aorta, where the affinity of prazosin appears to be about 10-fold higher than in most other tissues (16, 78, 216, 282) (see data compilation in ref. 244).

Drew (95) suggested that pA_2 values reported for yohimbine in blocking responses in various isolated tissues,

which were highly sensitive to prazosin blockade and therefore presumably mediated by α_1-adrenergic receptors, could be divided into different subgroups. He suggested that there might exist two different subtypes of α_1-adrenergic receptors differentiated by their affinities for yohimbine. In reviewing data from their own and other laboratories, Flavahan and Vanhoutte (105) noted that the wide variation in affinity for prazosin reported in functional studies in different tissues was reflected by a similar wide variation in affinity for yohimbine, even though none of the tissues studied appeared to contain postjunctional α_2-adrenergic receptors (fig. 9). They proposed that the data they had compiled suggested that there were two distinct subtypes of α_1-adrenergic receptors which could be distinguished by their affinities for both prazosin and yohimbine. One subtype had a high affinity for both drugs (pA_2s greater than 9 for prazosin and greater than 6.4 for yohimbine), and the other had a low affinity for both drugs (pA_2s less than 9 and 6.2, respectively).

Binding studies have generally provided little support for this proposal. Radioligand binding studies using [^{3}H] prazosin have usually suggested that prazosin has similar affinities for α_1-adrenergic receptors in all tissues examined. Bylund (48) recently compiled K_d values for [^{3}H] prazosin (pK_d) in binding to α_1-adrenergic receptors in 25 different tissues from five different species. pK_d values were generally around 9.3 to 9.7, similar to the high affinity subtype proposed above. There was little evidence for the existence of a low affinity subtype in any of these tissues since Scatchard plots were generally linear, indicative of a single class of binding sites. Calculation of binding constants by kinetic analysis gave similar results (48). These data would suggest that this

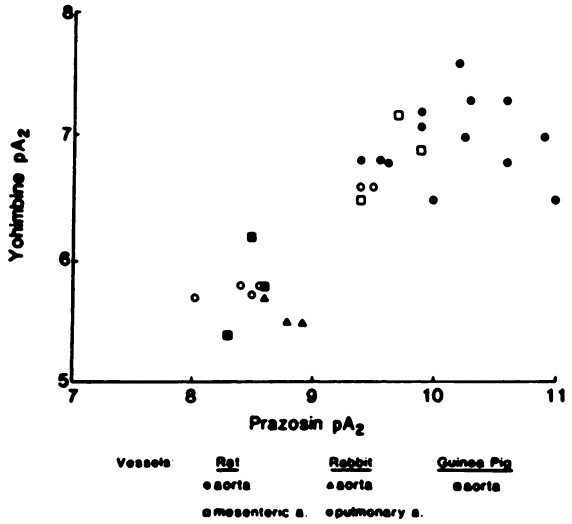


FIG. 9. Correlation between the potencies of prazosin and yohimbine in blocking α_1-adrenergic receptor-mediated contractions in isolated blood vessels. The grouping of the data into two general sets may suggest the existence of different receptor subtypes with different affinities for both antagonists. From Flavahan and Vanhoutte (105) with permission.

"high affinity" subtype would predominate in most tissues, while functional studies suggest that this subtype occurs mainly in rat aorta and mesenteric artery, and that the "low affinity" subtype predominates in most other tissues (2, 105, 244). Two binding studies have been performed on rat aorta. Descombes and Stoclet (82) found that [^{3}H]prazosin labeled α_1-adrenergic receptor binding sites in rat aorta with a pK_d of 9.4, which was similar to its affinity for the receptors in bovine aorta, as well as in most other tissues (48). Jones et al. (173) showed that [^{3}H]prazosin labeled α_1-adrenergic receptors in membranes from rat aorta with a pK_d of 9.7 in Tris buffer, but that this value was increased to 10.6 in glycylglycine buffer. Similar data were obtained in the dog aorta (173). Thus these binding studies do not support the proposition that the α_1-adrenergic receptors in rat aorta have a substantially higher affinity for prazosin than the α_1-adrenergic receptors in many other tissues.

Recently, however, two reports have appeared which support the existence of two distinct affinity constants for prazosin in radioligand binding studies. Babich et al. (11) examined the binding of [^{3}H]prazosin to microsomal preparations from rabbit aorta. They reported a slight curvature in the Scatchard plot. Computer analysis resulted in a significant two-site fit, with low and high affinity pK_ds of 10.3 and 9, respectively. Few conclusions could be drawn about the low affinity site, however, since the highest [^{3}H]prazosin concentration examined was 1.2 nM, which would result in only about a 50% saturation of this site. Jagadeesh and Deth (160) recently reported that competition of unlabeled prazosin for [^{3}H] prazosin binding sites in purified membranes from rat aorta was characterized by a low Hill coefficient. Computer modeling suggested the existence of two different affinity states for prazosin, although with less than 10-fold affinity differences (pK_ds of 9.8 and 10.6). However, extensive direct saturation analysis of [^{3}H]prazosin binding showed no evidence for binding site heterogeneity. The Scatchard plot was linear from 5 to 98% occupancy, with a correlation coefficient of 0.997 (160). The authors suggested that the relatively low specific activity of [^{3}H] prazosin might have prevented detection of high affinity binding sites. This could also be explained if [^{3}H]prazosin differed from unlabeled prazosin. However, the potency of prazosin in competing for α_1-adrenergic receptor binding sites labeled by either [^{3}H]prazosin or ^{125}IBE also does not vary substantially between different tissues (48, 244), and K_i values are very similar to K_d values determined for [^{3}H]prazosin directly by Scatchard analysis. In addition, the two affinity constants reported in these studies (11, 160) for prazosin do not agree with the data from functional studies. The low affinity binding site (pK_d 9.0 to 9.5) is similar to the high affinity functional receptor (pA_2 greater than 9.0; 105) and different from the low affinity functional receptor (pA_2 less than 9.0). Thus, the available radioligand binding data provide

little support for the existence of subtypes of α_1-adrenergic receptors with different affinities for prazosin.

2. WB 4101. On the other hand, radioligand binding studies have provided direct evidence for subdivision of α_1-adrenergic receptor binding sites based on their affinities for the antagonists WB 4101 and phentolamine. Battaglia et al. (13) first reported that inhibition of [^{3}H]prazosin binding in rat frontal cortex by both WB 4101 and phentolamine was characterized by low Hill coefficients. This was in contrast to a variety of other antagonists, including both prazosin and yohimbine, where the Hill coefficients were approximately 1.0. It was also in contrast to the inhibition of [^{3}H]prazosin binding in porcine pituitary, where both WB 4101 and phentolamine had Hill coefficients close to 1.0, just like the other antagonists. Interestingly, WB 4101 was approximately 15-fold more potent in competing for [^{3}H]prazosin binding sites in pituitary than in cortex, although phentolamine had similar potencies in both tissues (13). These authors raised the possibility that there might be pharmacological differences in the α_1-adrenergic receptor binding sites in these tissues.

These observations were confirmed by Morrow et al. (253), who showed that the curves for WB 4101 and phentolamine in competing for [^{3}H]prazosin binding in membranes from rat cerebral cortex were significantly better fit by a two-site rather than a one-site model (fig. 10). These authors suggested that there were two subtypes of α_1-adrenergic receptor binding sites in rat cerebral cortex, with equal affinities for prazosin and most other antagonists, but with different affinities for WB 4101 and phentolamine. WB 4101 was slightly more selective than phentolamine, with pK_ds of 9.2 and 7.6 at the two different binding sites (253). Morrow and Creese (254) compared the binding of [^{3}H]prazosin to the binding of [^{3}H]WB 4101 in membranes from rat hippocampus. They showed, as had been shown previously by others (286), that [^{3}H]WB 4101 labeled two binding sites, but that the low affinity site appeared to be serotonergic

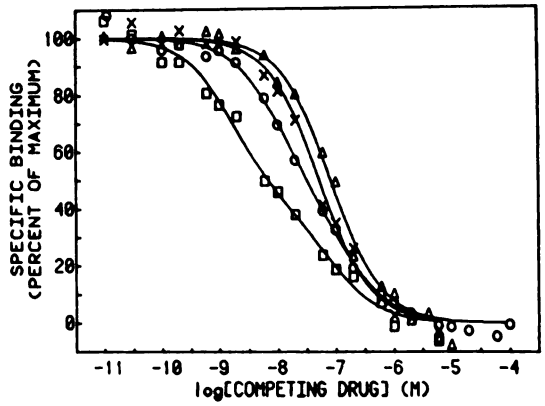


FIG. 10. Heterogeneity in the competition of antagonists for [^{3}H]prazosin binding sites in membranes from rat cerebral cortex. Only WB 4101 (□) and to a lesser extent phentolamine (○) show evidence for two affinity components. From Morrow and Creese (254) with permission.

in nature. When this site was eliminated, the density of [^{3}H]WB 4101 binding sites was about half that of [^{3}H]prazosin binding sites, and the pK_d for [^{3}H]WB 4101 (9.5) was similar to that for the high affinity binding site discussed above (254). Reviewing the literature, Morrow and Creese (254) suggested that α_1-adrenergic receptor binding sites and responses could be subdivided on the basis of the ratio of their relative affinities for the antagonists phentolamine and prazosin. These ranged from a low of 3.1 in rat submandibular gland (49) to a high of 381 in guinea pig heart (178) with a wide range of intermediate values. Morrow and Creese (254) suggested that the two types of α_1-adrenergic receptor binding sites should be called α_{1A} and α_{1B}. Jagadeesh and Deth (160) reported similar heterogeneity in the inhibition of [^{3}H]prazosin by WB 4101 in membranes from bovine aorta.

Using a different approach which eventually converged with the above results, Johnson and Minneman (169) noted differences in the sensitivity of α_1-adrenergic receptor binding sites to inactivation by the alkylating derivative of clonidine, chlorethylclonidine (202) (CEC). In attempting to differentiate between the α_1-adrenergic receptors increasing cyclic AMP and inositol phosphate accumulation in rat cerebral cortex, these authors noted that pretreatment of membranes with CEC dose-dependently inactivated only approximately half of the α_1-adrenergic receptor binding sites. Further increasing the concentration of CEC by 100-fold caused no further inactivation. This was in contrast to other alkylating agents, including phenoxybenzamine, dibenamine, and benextramine, which completely inactivated all α_1-adrenergic receptor binding sites in this tissue in a monophasic manner (169, 240). Examination of the effect of CEC on other brain regions showed that CEC pretreatment did not inactivate any α_1-adrenergic receptor binding sites in membranes from hippocampus (169). These results suggested that there were two distinct types of α_1-adrenergic receptor binding sites in rat cerebral cortex, only one of which could be inactivated by CEC. Rat hippocampus appeared to contain only the type of receptor insensitive to CEC inactivation. Comparing the effect of CEC on α_1-adrenergic receptor-stimulated inositol phosphate and cyclic AMP accumulation in cerebral cortex, however, gave inconclusive results (169). High concentrations of CEC (100 μM) were necessary to obtain significant inactivation in slice preparations. This high concentration of CEC partially blocked the cyclic AMP response but had no significant effect on the inositol phosphate response. Although these results raised the possibility that the receptors mediating these two responses might be differentially affected by CEC, since only small effects were seen at high concentrations, they were difficult to interpret conclusively.

If different subtypes of α_1-adrenergic receptors were differentially sensitive to CEC, then it might be possible to find tissues containing predominantly only a single

subtype. In screening a variety of tissues, Han et al. (130) showed that pretreatment of membranes from rat liver or spleen with 10 μM CEC caused a 70 to 80% loss of specific ^{125}IBE binding sites, but that this treatment had no effect on ^{125}IBE binding sites in membranes from rat hippocampus or vas deferens. Interestingly, binding studies showed that CEC did not appear to have different affinities in binding to the receptors in these different tissues; the difference appeared to lie in the ability of CEC to inactivate the receptors (130). These results suggested that rat liver and spleen contained mainly "CEC-sensitive" α_1-adrenergic receptors, while hippocampus and vas deferens contained mainly "CEC-insensitive" receptors. These findings were important for two reasons. First, contractile responses to α_1-adrenergic receptor stimulation could be measured in one of each of the CEC-sensitive (spleen) and CEC-insensitive (vas deferens) tissues, allowing comparison of results from radioligand binding studies to functional studies on receptor activation. The selectivity of CEC in binding studies was shown to clearly extend to functional receptor activation. Pretreatment of intact tissues with 100 μM CEC for 30 min greatly reduced the contractile response to norepinephrine in rat spleen, without affecting the response in vas deferens (fig. 11) (130). In addition, these tissues could be used to easily screen for other pharmacological differences between the proposed receptor subtypes, particularly the affinities of competitive antagonists.

In comparing the potencies of a variety of agonists and antagonists in competing for ^{125}IBE binding sites in CEC-sensitive (liver) and insensitive (hippocampus) tissues, Han et al. (130) showed that several drugs appeared to have different potencies at the putative receptor subtypes. The most selective compound appeared to be the imidazoline agonist oxymetazoline, which was 9-fold more potent in hippocampus than liver, followed closely by WB 4101 and its congener benoxathian, which were 6- to 7-fold more potent in hippocampus than liver. Phentolamine also showed small (3-fold) differences in potency. Similar, although smaller, differences in potency were observed when α_1-adrenergic receptor binding sites in spleen and vas deferens were compared (130). The Hill coefficients for these apparently selective competitive antagonists were close to 1.0 in liver and spleen, consistent with the existence of a single receptor subtype predicted by the almost complete inactivation observed with CEC in these tissues. Surprisingly, however, the Hill coefficients for the selective (but not the nonselective) antagonists were substantially less than 1.0 in hippocampus and vas deferens, suggesting a possible heterogeneity in the receptor populations in these tissues (130). This was in apparent contradiction to the almost complete insensitivity to CEC inactivation by the receptors in these tissues.

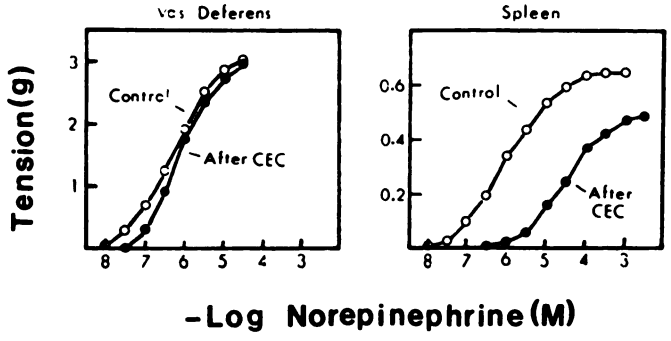


FIG. 11. Effect of CEC on norepinephrine-induced contractions of rat vas deferens (α_{1a}) and spleen (α_{1b}). The selective antagonism of the contractile response in spleen supports the selectivity of this drug in inactivating the α_1-adrenergic receptors observed in radioligand binding assays. From Han et al. (130) with permission.

Computer-assisted analysis of WB 4101 and benoxathian competition curves showed that a two-site fit was substantially better than a one-site fit in the hippocampus and vas deferens (131) with both sites existing in approximately equal proportions, in agreement with previous results in the cerebral cortex and hippocampus (253, 254). Liver and spleen appeared to contain only a single type of binding site, and the affinity of both WB 4101 and benoxathian for this site in liver and spleen agreed well with the affinity for the relatively low affinity site in hippocampus and vas deferens. These data suggested that hippocampus and vas deferens contained both α_{1a}- and α_{1b}-type binding sites, while liver and spleen had only the α_{1b}- subtype, as defined by Morrow and Creese (254). To determine whether these differences in binding sites were reflected in the functional receptors, the potencies of these antagonists in blocking α_1-adrenergic receptor-mediated contractile responses in rat spleen and vas deferens were determined. Both WB 4101 and benoxathian were 10- to 20-fold more potent in blocking contractions of vas deferens (containing both subtypes) than in blocking contractions of spleen (containing only the α_{1b}-subtype). The pA_2 values calculated in the vas deferens agreed well with the pK_d values for the α_{1a}-subtype, while pA_2 values obtained in the spleen agreed well with the pK_d values for the α_{1b}-subtype (131). These results suggested that the binding sites differentiated by WB 4101 represented true receptor subtypes, and that activation of both subtypes could activate contractile responses in different tissues. Although binding studies showed that the rat vas deferens contained both types of receptors, only the α_{1a} subtype appeared to be involved in the contractile response to norepinephrine. The contractile response of rat spleen to norepinephrine was mediated by the α_{1b}-subtype, which was the only subtype found in the tissue with binding studies.

Taken together, the similarity between the results from both binding and functional studies strongly suggested the existence of two subtypes of α_1-adrenergic receptors with different sensitivities to CEC and different affinities for the competitive antagonists WB 4101 and benoxathian. However, despite the general correla-

tion between CEC sensitivity and the proportion of low affinity WB 4101 binding sites (α_{1b}) in each tissue, there was not quantitative agreement between these two approaches. In all tissues, a substantially greater proportion of the receptors had a low affinity for WB 4101 than could be inactivated by CEC. Although both hippocampus and vas deferens appeared to contain equal proportions of the two subtypes defined with WB 4101 (131), there was no effect of CEC inactivation on the binding sites in these tissues (130). Similarly, although liver and spleen appeared to contain only the α_{1b}-subtype as defined with WB 4101 (131), 20 to 30% of the binding sites were resistant to inactivation by CEC (130).

Further studies showed that this discrepancy was probably caused by incomplete inactivation by CEC, a highly water-soluble compound, under the conditions studied (246). When membranes from rat hippocampus were repetitively exposed to four separate incubations with 10 μM CEC, 25% of the binding sites were inactivated. These results suggested that CEC might not be able to gain access to all the α_1-adrenergic receptors in a single incubation period. All CEC inactivation experiments had been performed in phosphate-buffered saline which is routinely used for ^{125}IBE binding experiments. Since such isotonic conditions promote resealing of vesicles following tissue homogenization, the high water solubility of CEC might have prevented it from crossing lipid bilayers to gain access to binding sites enclosed in such vesicles. Therefore the ability of CEC to inactivate ^{125}IBE binding sites in a hypotonic buffer (10 mM sodium Hepes) which would promote vesicle lysis was tested. CEC caused a much greater inactivation of ^{125}IBE binding sites under such hypotonic conditions in all tissues examined than had been observed under the isotonic conditions used previously (246). In the presence of isotonic NaCl, 10 μM CEC had no effect on the density of sites in hippocampus, while under hypotonic conditions this treatment caused a 41% loss in binding sites. Similar increases in inactivation were observed in all other tissues (246). When the proportion of α_1-adrenergic receptor binding sites with a low affinity for WB 4101 was compared to the proportion sensitive to CEC inactivation under hypotonic conditions, an excellent correlation was observed. The best fit line was close to the line of identity, suggesting that these two methods are distinguishing the same subpopulations of binding sites. Finally, when WB 4101 inhibition curves were examined following pretreatment of membranes from hippocampus or vas deferens with CEC, the apparent low affinity sites had been eliminated (246).

These results suggest that two subtypes of α_1-adrenergic receptors can be differentiated by the competitive antagonist WB 4101 and the alkylating agent CEC. The α_{1a} subtype has a high affinity for WB 4101 (pK_d 9.4) and is not inactivated by CEC in concentrations up to 100 μM. The α_{1b} subtype has a 10- to 20-fold lower affinity for WB 4101 (pK_d 8.0) and is inactivated by CEC with an EC_{50} around 1 μM. The relationship of these receptor subtypes to those differentiated by prazosin, yohimbine, or selective agonists is not yet clear. Both prazosin and yohimbine appear to have similar affinities for both of these subtypes (131) (pK_ds of 9 and 6, respectively). Although oxymetazoline appears to distinguish between these receptors (pK_ds of 8 and 6.8), clonidine and other imidazolines appear to have similar affinities for both subtypes. The actual interrelationships between these studies will need to be clarified by further experimental work.

XI. Relationship to Inositol Phospholipid Metabolism and Ca^{2+} Influx

Once strong evidence for pharmacologically distinct α_1-adrenergic receptor subtypes was obtained, one of the first interesting questions to be answered is whether they utilize the same signal transduction mechanism. The availability of specific and well-characterized drugs which clearly distinguish between different receptor subtypes makes it relatively easy to examine the possibilities that different receptor subtypes are linked to the alternate signal transduction mechanisms discussed above.

The marked variations in the importance of extracellular Ca^{2+} for α_1-adrenergic receptor-mediated contraction of smooth muscle raise the obvious possibility that one receptor subtype might release intracellular Ca^{2+}, while the other might promote Ca^{2+} influx (147, 228) (see above). As discussed above, Korstanje et al. (191) and Beckeringh et al. (16) showed that several competitive antagonists had similar potencies in blocking both Ca^{2+} influx-dependent and independent responses to α_1-adrenergic receptor agonists, concluding that the same receptor subtypes were involved in both processes. However, neither WB 4101 nor CEC was examined in these studies. When both binding and functional studies suggested the existence of α_{1a}- and α_{1b}-adrenergic receptors differentiated by these two compounds, it was of interest to examine the role of Ca^{2+} influx in the contractile responses initiated by each subtype. Norepinephrine-induced contractions of rat vas deferens appear to be mediated solely by α_{1a}-adrenergic receptors, since the pA_2 for WB 4101 is about 9.3 and the contractile response is completely unaffected by pretreatment with 100 μM CEC (130, 131). Conversely, norepinephrine-mediated contractions of rat spleen appear to be mediated solely by α_{1b}-adrenergic receptors, since they are blocked by WB 4101 with a pA_2 of 8.0 and are markedly antagonized by CEC pretreatment. Han et al. (131) showed that 1 μM nifedipine markedly reduced the contractile response of vas deferens (α_{1a}) but did not affect the contractile response of spleen (α_{1b}) (fig. 12). In the presence of nifedipine, a residual contractile response was observed in vas deferens, but this response was much less sensitive to inhibition by WB 4101 (131), suggesting that the α_{1b}-adrenergic receptor population, which binding studies

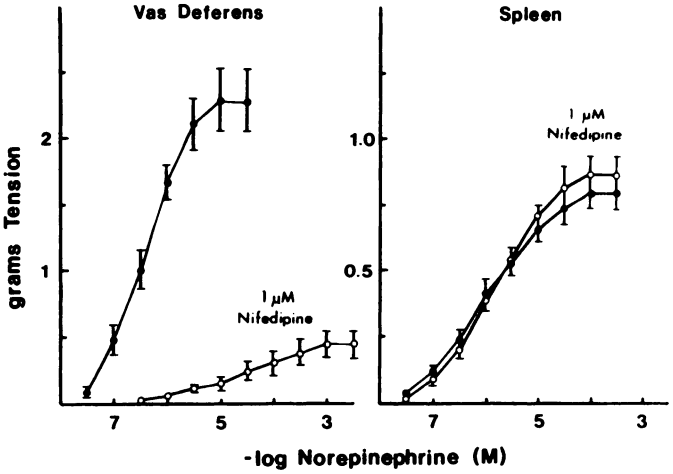


FIG. 12. Effect of nifedipine on norepinephrine-induced contractions of rat vas deferens (α_{1a}) and spleen (α_{1b}). The selective antagonism of the contractile response in vas deferens supports the possibility that α_{1a}-adrenergic receptors may alter the permeability of dihydropyridine-sensitive Ca^{2+} channels. From Han et al. (131) with permission. *Bars*, SE.

show to exist in this tissue, can mediate contraction when the other subtype is functionally neutralized. Similarly, chelation of extracellular Ca^{2+} with EGTA almost abolished the contractile response of vas deferens, but had no effect in spleen (131).

These studies clearly resurrect the intriguing possibility that Ca^{2+} influx-dependent and independent contractile responses to α_1-adrenergic receptor activation are mediated by pharmacologically distinct receptor subtypes. We hypothesized that activation of α_{1a}-adrenergic receptors may primarily activate Ca^{2+} influx through dihydropyridine-sensitive channels, while activation of α_{1b}-adrenergic receptors may cause release of stored intracellular Ca^{2+} (131). However, many more tissues need to be examined before unequivocal conclusions can be drawn. Unfortunately, we have found that in many tissues contractile responses to norepinephrine appear to involve both α_1-adrenergic receptor subtypes to differing degrees. We seem to have been fortunate in happening upon the clear differentiation between vas deferens and spleen, where responses appear to be mediated by only a single receptor subtype. In general, this appears to be the exception rather than the rule (P. W. Abel and K. P. Minneman, unpublished data).

If this hypothesis is correct, one would predict that only α_{1b}-adrenergic receptors would be linked to hydrolysis of PIP_2 and formation of $Ins(1,4,5)P_3$, since it is this latter compound that releases stored intracellular Ca^{2+}. The evidence we have obtained to date supports this hypothesis. Han et al. (131) determined pA_2 values for WB 4101 in blocking norepinephrine-stimulated [^{3}H]inositol phosphate accumulation in the presence of lithium in tissues which, from radioligand binding assays, were known to contain both α_1-adrenergic receptor subtypes. The pA_2 values ranged from 8 to 8.3 in all tissues examined, suggesting that only α_{1b}-adrenergic receptors were activating this response. In fact, the pA_2 for WB 4101 in blocking norepinephrine-stimulated [^{3}H]inositol phosphate accumulation in the rat vas deferens (108) was found to be 8.2, even though the pA_2 for this drug in blocking the contractile response in this tissue was 9.3 (131). Thus it appeared that, in this tissue which contained both receptor subtypes, α_{1b}-adrenergic receptors were activating inositol phosphate formation but α_{1a}-adrenergic receptors were activating the contractile response by promoting Ca^{2+} influx through nifedipine-sensitive channels. Similarly, CEC pretreatment markedly reduced norepinephrine-stimulated [^{3}H]inositol phosphate accumulation in the rat vas deferens (246) without affecting the contractile response of this tissue (130). Finally, CEC pretreatment of rat liver slices, which contain only the α_{1b} subtype, caused a parallel inactivation of receptor binding sites and norepinephrine-stimulated inositol phosphate accumulation (246). These results are all consistent with the hypothesis that only the α_{1b}-adrenergic receptor subtypes are linked to inositol phosphate accumulation in these tissues.

It seems likely, however, that α_{1a}-adrenergic receptor activation might also activate inositol phospholipid metabolism in some tissues. The opening of dihydropyridine-sensitive Ca^{2+} channels, either by depolarization or application of Ca^{2+} channel agonist drugs, activates inositol phosphate formation in brain and other tissues (124, 185, 220, 291) (see above). If α_{1a}-adrenergic receptor activation promotes Ca^{2+} influx, either directly or indirectly, it is likely that this may also result in breakdown of inositol phospholipids (69). Possibly, there might be differential involvement of different inositol phosphates and/or diacylglycerol in signal transduction by the two subtypes. In particular, the differences between activation of phospholipase C by G protein activation and by increasing Ca^{2+} (69, 121) (see above) raise the possibility that activation of different receptor subtypes might increase inositol phospholipid metabolism by different mechanisms.

Another interesting possibility is that the α_1-adrenergic receptors activating phospholipase A_2 activity in various cells (45, 46, 142, 318) may be of a different subtype than those activating phospholipase C activity. This would explain the differential sensitivities of these two processes to toxins and drugs (45). Also, if arachidonic acid release and prostaglandin formation are involved in the cyclic AMP response to α_1-adrenergic receptor stimulation (98, 268, 304) in the brain, the involvement of different receptors could explain the different abilities of agonists to activate inositol phosphate and cyclic AMP responses (168). All of these different processes, Ca^{2+}, inositol phosphates, diacylglycerol, cyclic AMP, cyclic GMP, arachidonic acid, membrane potential, and membrane channels, are clearly interrelated in complex manners in living cells, and the unambiguous dissection of these systems presents an interesting challenge. Clearly,

much work remains to be done in this area to distinguish the various signal transduction mechanisms activated by each receptor subtype.

XII. Conclusions

It is now well established that activation of α_1-adrenergic receptors by norepinephrine or other agonists increases hydrolysis of PIP_2, probably through an intermediary G protein. The resulting release of $Ins(1,4,5)P_3$ and diacylglycerol into the intracellular milieu mobilizes Ca^{2+} stored in nonmitochondrial pools, such as the endoplasmic and sarcoplasmic reticulum, and activates protein kinase C. These primary events appear to be responsible for many of the effects of α_1-adrenergic receptor activation in many tissues.

However, it is increasingly clear that α_1-adrenergic receptors are not pharmacologically homogeneous. Evidence from radioligand binding studies, functional studies of smooth muscle contractile responses, and studies of receptor-mediated second messenger accumulation shows substantial differences in the affinities and efficacies of agonists, and the affinities of antagonists for α_1-adrenergic receptors in different tissues. Based on these differences, the existence of at least two discrete subtypes of α_1-adrenergic receptors seems clear. The best drugs currently available to distinguish between these receptor types appear to be the competitive antagonist WB 4101 and the irreversible alkylating agent chlorethylclonidine. However, it also seems likely that differences in the affinities and efficacies of imidazoline and phenethylamine agonists will be found as the two receptor subtypes are increasingly well characterized.

Similarly, much evidence now supports the contention that α_1-adrenergic receptor-mediated responses in some tissues may be mediated through a signal transduction mechanism unrelated to breakdown of PIP_2. Increases in Ca^{2+} influx, arachidonic acid release, cyclic AMP accumulation, and cyclic GMP accumulation have all been reported in response to α_1-adrenergic receptor activation, and some of these effects have been clearly differentiated from activation of inositol phospholipid breakdown. The possibility that different α_1-adrenergic receptor subtypes utilize different signal transduction mechanisms is increasingly attractive. A hypothesis consistent with most available data is that α_{1a}-adrenergic receptors primarily promote influx of extracellular Ca^{2+} through dihydropyridine-sensitive channels, while α_{1b}-adrenergic receptors initiate signals through the well-characterized inositol phospholipid pathway (fig. 13).

Overall, the existence of pharmacologically distinct subtypes of α_1-adrenergic receptors linked to different signal transduction mechanisms raises interesting questions concerning the basic mechanisms by which receptor activation controls the intracellular free Ca^{2+} concentration. Further characterization of these subtypes may also lead to the development of more selective and useful therapeutic agents for treating a variety of physiological disorders.

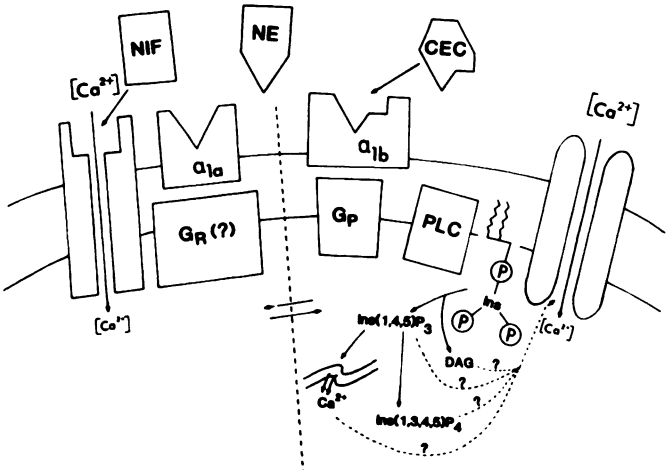


FIG. 13. Schematic diagram for different signal transduction mechanisms by two different α_1-adrenergic receptor subtypes. α_{1a}-Adrenergic receptors appear to activate phospholipase C (*PLC*) through a G protein (G_p) to cause hydrolysis of PIP_2 to $Ins(1,4,5)P_3$ and diacylglycerol (*DAG*). This may indirectly stimulate Ca^{2+} influx through non-dihydropyridine-sensitive channels. α_{1a}-Adrenergic receptors may, directly or indirectly, stimulate Ca^{2+} influx through nifedipine (*NIF*)-sensitive channels, possibly through a G protein (G_R). Chlorethylclonidine (*CEC*) selectively inactivates only the α_{1b} subtype, although norepinephrine (*NE*) activates both subtypes.

REFERENCES

1. AGHAJANIAN, G. K.: Modulation of a transient outward current in serotonergic neurones by α_1-adrenoceptors. Nature **315:** 501–503, 1985.
2. AGRAWAL, D. K., TRIGGLE, G. R., AND DANIEL, E. E.: Pharmacological characterization of the postsynaptic alpha-adrenoceptors in vascular smooth muscle from canine and rat mesenteric vascular beds. J. Pharmacol. Exp. Ther. **229:** 831–838, 1984.
3. AHLQUIST, R. P.: A study of the adrenotropic receptors. Am. J. Physiol. **153:** 586–600, 1948.
4. ALTIN, J. G., AND BYGRAVE, F. L.: The Ca^{2+}-mobilizing actions of vasopressin and angiotensin differ from those of the α-adrenergic agonist phenylephrine in the perfused rat liver. Biochem. J. **232:** 911–917, 1985.
5. AMBLER, S. K., AND TAYLOR, P.: Mobilization of intracellular calcium by α_1-adrenergic receptor activation in muscle cell monolayers. J. Biol. Chem. **261:** 5866–5871, 1986.
6. AMBLER, S. K., THOMPSON, B., SOLSKI, P. A., BROWN, J. H., AND TAYLOR, P.: Receptor-mediated inositol phosphate formation in relation to calcium mobilization: a comparison of two cell lines. Mol. Pharmacol. **32:** 376–383, 1987.
7. AMITAI, G., BROWN, R. D., AND TAYLOR, P.: The relationship between α_1-adrenergic receptor occupation and the mobilization of intracellular calcium. J. Biol. Chem. **259:** 12519–12527, 1984.
8. ARUNLAKSHANA, O., AND SCHILD, H. O.: Some quantitative uses of drug antagonists. Br. J. Pharmacol. **14:** 48–58, 1959.
9. ASTRACHAN, D. I., DAVIS, M., AND GALLAGHER, D. W.: Behavior and binding: correlations between α_1-adrenergic stimulation of acoustic startle and α_1-adrenoceptor occupancy and number in rat lumbar spinal cord. Brain Res. **260:** 81–90, 1983.
10. AWAD, R., PAYNE, R., AND DETH, R. C.: Alpha adrenergic receptor subtype associated with receptor binding, Ca^{2+} influx, Ca^{2+} release, and contractile events in the rabbit aorta. J. Pharmacol. Exp. Ther. **227:** 60–67, 1983.
11. BABICH, M., PEDIGO, N. W., BUTLER, B. T., AND PIASCIK, M. T.: Heterogeneity of alpha-1 receptors associated with vascular smooth muscle: evidence from functional and ligand binding studies. Life Sci. **41:** 663–673, 1987.
12. BALLA, T., GUILEMETT, G., BAUKAL, A. J., AND CATT, K. J.: Metabolism of inositol 1,3,4-trisphosphate to a new tetrakisphosphate isomer in angiotensin-stimulated adrenal glomerulosa cells. J. Biol. Chem. **262:** 9952–9955, 1987.
13. BATTAGLIA, G., SHANNON, M., BORGUNDVAAG, B., AND TITELER, M. Properties of ^{3}H-prazosin labeled α_1-adrenergic receptors in rat brain and porcine neurointermediate lobe tissue. J. Neurochem. **41:** 538–542, 1983.
14. BATTY, I. R., NAHORSKI, S. R., AND IRVINE, R. F.: Rapid formation of inositol 1,3,4,5-tetrakisphosphate following muscarinic receptor stimulation of rat cerebral cortex slices. Biochem. J. **232:** 211–215, 1985.
15. BEAN, B. P.: Two kinds of calcium channels in canine atrial cells. Differ-

ences in kinetics, selectivity, and pharmacology. J. Gen. Physiol. **86:** 1–30, 1985.
16. BECKERINGH, J. J., THOOLEN, M. J. M. C., DE JONGE, A., WILFFERT, B., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Differential effects of the calcium entry blocker D600 on contractions of rat and guinea pig aortas elicited by various alpha-1 adrenoceptor agonists. J. Pharmacol. Exp. Ther. **229:** 515–521, 1984.
17. BECKERINGH, J. J., THOOLEN, M. J. M. C., DE JONGE, A., WILFFERT, B., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: The contractions induced in rat and guinea pig aortic strips by the α_2-adrenoceptor selective agonists BHT 920 and UK 14,304 are mediated by α_1-adrenoceptors. Eur. J. Pharmacol. **104:** 197–203, 1984.
18. BENFEY, B. G.: Function of myocardial α-adrenoceptors. Life Sci. **31:** 101–112, 1982.
19. BENHAM, C. D., AND TSIEN, R. W.: A novel receptor-operated Ca^{2+} channel activated by ATP in smooth muscle. Nature (Lond.) **328:** 275–278, 1987.
20. BERRIDGE, M. J.: Phosphatidylinositol hydrolysis: a multi-functional transducing mechanism. Mol. Cell. Endocrinol. **24:** 115–140, 1981.
21. BERRIDGE, M. J.: Rapid accumulation of inositol trisphosphate reveals that agonists hydrolyse polyphosphoinositides instead of phosphatidylinositol. Biochem. J. **212:** 849–858, 1983.
22. BERRIDGE, M. J., DOWNES, C. P., AND HANLEY, M. R.: Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem. J. **206:** 587–595, 1982.
23. BERTHELSEN, S., AND PETTINGER, W. A.: A functional basis for the classification of α-adrenergic receptors. Life Sci. **21:** 595–606, 1977.
24. BESSE, J. C., AND FURCHGOTT, R. F.: Dissociation constants and relative efficacies of agonists acting on α-adrenergic receptors in rabbit aorta. J. Pharmacol. Exp. Ther. **197:** 66–78, 1976.
25. BEVAN, J. A.: A comparison of the contractile responses of the rabbit basilar and pulmonary arteries to sympathomimetic agonists: further evidence for variation in vascular adrenoceptor characteristics. J. Pharmacol. Exp. Ther. **216:** 83–89, 1981.
26. BEVAN, J. A.: Diltiazem selectively inhibits cerebrovascular extrinsic but not intrinsic myogenic tone. Circ. Res. **52** (suppl. I): 104–109, 1983.
27. BEVAN, J. A., BEVAN, R. D., HWA, J. J., OWEN, M. P., TAYO, F. M., AND WINQUIST, R. J.: Calcium extrinsic and intrinsic (myogenic) vascular tone. *In* Calcium Modulators, eds. by T. Godfraind, A. Albertini, and R. Paoletti, pp. 125–132, Elsevier, Amsterdam, 1982.
28. BEVAN, J. A., OBIOWO, M. A., AND BEVAN, R. D.: Physiological variation in α-adrenoceptor mediated arterial sensitivity: relation to agonist affinity. Science (Wash. DC) **234:** 196–197, 1986.
29. BLACKMORE, P. F., DEHAYE, J.-P., AND EXTON, J. H.: Studies on α-adrenergic activation of hepatic glucose output: the role of mitochondrial calcium release in α-adrenergic activation of phosphorylase in perfused rat liver. J. Biol. Chem. **254:** 6945–6950, 1979.
30. BLACKMORE, P. R., WAYNICK, L. E., BLACKMAN, G. E., GRAHAM, C. W., AND SHERRY, R. S.: α- and β-Adrenergic stimulation of parenchymal cell Ca^{2+} influx: influence of extracellular pH. J. Biol. Chem. **259:** 12322–12325, 1984.
31. BOHM, M., SCHMITZ, W., AND SCHOLZ, H.: Evidence against a role of a pertussis toxin sensitive guanine nucleotide binding protein in the α_1-adrenoceptor mediated positive inotropic effect in the heart. Naunyn Schmiedebergs Arch. Pharmakol. **335:** 476–479, 1987.
32. BOHR, D. F.: Vascular smooth muscle: dual effect of calcium. Science (Wash. DC) **139:** 579–599, 1963.
33. BOHR, D. F.: Vascular smooth muscle update. Circ. Res. **32:** 665–672, 1973.
34. BOLTON, T. B.: Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. **59:** 606–718, 1979.
35. BORLE, A. B.: Control, modulation, and regulation of cell calcium. Rev. Physiol. Biochem. Pharmacol. **90:** 13–153, 1981.
36. BOU, J., LLENAS, J., AND MASSINGHAM, R.: Calcium entry blocking drugs, calcium antagonists, and vascular smooth muscle function. J. Auton. Pharmacol. **3:** 219–232, 1983.
37. BOU, J., AND MASSINGHAM, R.: Effect of diltiazem upon contractile responses to phenylephrine, cirazoline, Sgd 101/75, and B-HT 920 in rabbit aorta and dog saphenous vein preparations. Eur. J. Pharmacol. **121:** 319–325, 1986.
38. BOYER, J. L., GARCIA, A., POSADAS, C., AND GARCIA-SAINZ, J. A.: Differential effect of pertussis toxin on the affinity state of renal α_1- and α_2-adrenoceptors. J. Biol. Chem. **259:** 8076–8079, 1984.
39. BRADFORD, P. G., AND RUBIN, R. P.: Guanine nucleotide regulation of phospholipase C activity in permeabilized rabbit neutrophils: inhibition by pertussis toxin and sensitization to submicromolar calcium concentrations. Biochem. J. **239:** 97–102, 1986.
40. BROADLEY, K. J.: Cardiac adrenoceptors. J. Autonom. Pharmacol. **2:** 119–145, 1982.
41. BROWN, A. M., AND BIRNBAUMER, L.: Direct G protein gating of ion channels. Am. J. Physiol. **254:** H401–H410, 1988.
42. BROWN, E., KENDALL, D. A., AND NAHORSKI, S. R.: Inositol phospholipid hydrolysis in rat cerebral cortical slices. I. Receptor characterization. J. Neurochem. **42:** 1379–1387, 1984.
43. BROWN, J. H., BUXTON, I. L., AND BRUNTON, L. L.: α_1-Adrenergic and muscarinic cholinergic stimulation of phosphoinositide hydrolysis in adult rat cardiomyocytes. Circ. Res. **57:** 532–537, 1985.
44. BULBRING, E., AND TOMITA, T.: Catecholamine action on smooth muscle. Pharmacol. Rev. **39:** 49–96, 1987.
45. BURCH, R. M., LUINI, A., AND AXELROD, J.: Phospholipase A_2 and phospholipase C are activated by distinct GTP binding proteins in response to α_1-adrenergic stimulation in FRTL5 thyroid cells. Proc. Natl. Acad. Sci. USA **83:** 7201–7205, 1986.
46. BURCH, R. M., LUINI, L. A., MAIS, D. E., CORDA, D., VANDERHOEK, J. Y., KOHN, L. D., AND AXELROD, J.: α_1-Adrenergic stimulation of arachidonic acid release and metabolism in a rat thyroid cell line: mediation of cell replication by prostaglandin E_2. J. Biol. Chem. **261:** 11236–11241, 1986.
47. BUXTON, I. L. O., AND BRUNTON, L. L.: Action of the cardiac α_1-adrenergic receptor: activation of cyclic AMP degradation. J. Biol. Chem. **260:** 6733–6737, 1985.
48. BYLUND, D. B.: Biochemistry and pharmacology of the alpha-1 adrenergic receptor. *In* The Alpha-1 Adrenergic Receptors, ed. by R. R. Ruffolo, Jr., pp. 19–69, Humana Press, Clifton, NJ, 1987.
49. BYLUND, D. B., MARTINEZ, J. R., AND PICKLE, D. L.: Regulation of autonomic receptors in rat submandibular gland. Mol. Pharmacol. **21:** 27–35, 1982.
50. CAMBRIDGE, D., DAVEY, M. J., AND MASSINGHAM, R.: Prazosin, a selective antagonist of postsynaptic α-adrenoceptors. Br. J. Pharmacol. **59:** 514P, 1977.
51. CAMPBELL, M. D., DETH, R. C., PAYNE, R. A., AND HONEYMAN, T. W.: Phosphoinositide hydrolysis is correlated with agonist-induced calcium flux and contraction in the rabbit aorta. Eur. J. Pharmacol. **116:** 129–136, 1985.
52. CASTEELS, R., AND DROOGMANS, G.: Exchange characteristics of the noradrenaline sensitive calcium store in vascular smooth muscle cells of rabbit ear artery. J. Physiol. **317:** 263–279, 1981.
53. CAUVIN, C., LOUTZENHISER, R., HWANG, O., AND VAN BREEMEN, C.: α_1-Adrenoceptors induce Ca influx and intracellular Ca release in isolated rabbit aorta. Eur. J. Pharmacol. **84:** 233–235, 1982.
54. CAUVIN, C., LOUTZENHISER, R., AND VAN BREEMAN, C.: Mechanisms of calcium antagonist-induced vasodilation. Annu. Rev. Pharmacol. Toxicol. **23:** 373–396, 1983.
55. CAUVIN, C., AND MALIK, S.: Induction of Ca^{2+} influx and intracellular Ca^{2+} release in isolated rat aorta and mesenteric resistance vessels by norepinephrine activation of alpha-1 receptors. J. Pharmacol. Exp. Ther. **230:** 413–418, 1984.
56. CAVERO, I., SHEPPERSON, N., LEFEVRE-BORG, F., AND LANGER, S. Z.: Differential inhibition of vascular smooth muscle responses to α_1- and α_2-adrenoceptor agonists by diltiazem and verapamil. Circ. Res. **52** (suppl. 1): 69–76, 1983.
57. CHAN, T. M., BLACKMORE, P. F., STEINER, K. E., AND EXTON, J. H.: Effects of adrenalectomy on hormone action on hepatic glucose metabolism. J. Biol. Chem. **254:** 2428–2433, 1979.
58. CHAN, T. M., AND EXTON, J. H.: α-Adrenergic-mediated accumulation of adenosine 3′,5′-monophosphate in calcium depleted hepatocytes. J. Biol. Chem. **252:** 8645–8653, 1977.
59. CHASIN, M., MAMRAK, F., AND SAMANIEGO, S. G.: Preparation and properties of a cell-free hormonally responsive adenylate cyclase from guinea pig brain. J. Neurochem. **22:** 1031–1038, 1974.
60. CHIU, A. T., BOZARTH, J. M., AND TIMMERMANS, P. B. M. W. M.: Relationship between phosphatidylinositol turnover and Ca^{2+} mobilization induced by alpha-1 adrenoceptor stimulation in the rat aorta. J. Pharmacol. Exp. Ther. **240:** 123–127, 1987.
61. CHIU, A. T., MCCALL, D. E., THOOLEN, M. J. M. C., AND TIMMERMANS, P. B. M. W. M.: Ca^{2+} utilization in the constriction of rat aorta to full and partial alpha-1 adrenoceptor agonists. J. Pharmacol. Exp. Ther. **238:** 224–231, 1986.
62. COATES, J., JAHN, U., AND WEETMAN, D. F.: The existence of a new subtype of α-adrenoceptor on the rat anococcygeus is revealed by Sgd 101/75 and phenoxybenzamine. Br. J. Pharmacol. **75:** 549–552, 1982.
63. COATES, J., AND WEETMAN, D. F.: Demonstration of α_{1a}-adrenoceptors after exposure of the rat anococcygeus to benextramine. Br. J. Pharmacol. **80:** 343–346, 1983.
64. COCKROFT, S., AND GOMPERTS, B. D.: Role of guanine nucleotide binding protein in the activation of polyphosphoinositidephosphodiesterase. Nature (Lond.) **314:** 534–536, 1985.
65. COLUCCI, W. S., BROCK, T. A., GIMBRONE, M. A., JR., AND ALEXANDER, R. W.: Nonlinear relationship between α_1-adrenergic receptor occupancy and norepinephrine-stimulated calcium flux in cultured vascular smooth muscle cells. Mol. Pharmacol. **27:** 517–524, 1985.
66. COLUCCI, W. S., GIMBRONE, M. A., JR., AND ALEXANDER, R. W.: Regulation of myocardial and vascular α-adrenergic receptor affinity. Effects of guanine nucleotides, cations, estrogen, and catecholamine depletion. Circ. Res. **55:** 78–88, 1984.
67. COOKE, J. P., RIMELE, T. J., FLAVAHAN, N. A., AND VANHOUTTE, P. M.: Nimodipine and inhibition of alpha adrenergic activation of the isolated canine saphenous vein. J. Pharmacol. Exp. Ther. **234:** 598–603, 1985.
68. CORVERA, S., HERNANDEZ-SOTOMAYOR, S. M. T., AND GARCIA-SAINZ, J. A.: Modulation by thyroid status of cyclic AMP dependent and calcium dependent mechanisms of hormone action in rat liver cells: possible involvement of two different signal transduction mechanisms in α_1-adrenergic action. Biochim. Biophys. Acta **803:** 95–105, 1984.

69. CREWS, F. T., GONZALES, R. A., RAULLI, R., MCELHANEY, R., PONTZER, N., AND RAIZADA, M.: Interaction of calcium with receptor-stimulated phosphoinositide hydrolysis in brain and liver. Ann. NY Acad. Sci. **522:** 88–95, 1988.
70. CUBEDDU, L., BARNES, E. M., LANGER, S. Z., AND WEINER, N.: Release of norepinephrine and dopamine-β-hydroxylase by nerve stimulation. I. Role of neuronal and extraneuronal uptake and of α presynaptic receptors. J. Pharmacol. Exp. Ther. **190:** 431–450, 1974.
71. DALRYMPLE, H. W., HAMILTON, C. A., AND REID, J. L.: Alpha adrenoceptors and contractile responses in isolated arterial smooth muscle. Br. J. Pharmacol. **74:** 264, 1981.
72. DALY, J. W., MCNEAL, E., PARTINGTON, C., NEUWIRTH, M., AND CREVELING, C. R.: Accumulations of cyclic AMP in adenine-labeled cell-free preparations from guinea pig cerebral cortex: role of α-adrenergic and H_1-histaminergic receptors. J. Neurochem. **35:** 326–337, 1980.
73. DALY, J. W., PADGETT, W., CREVELING, C. R., CANTACUZENE, D., AND KIRK, K. L.: Fluoronorepinephrines: specific agonists for the activation of alpha and beta adrenergic sensitive cyclic AMP generating systems in brain slices. J. Pharmacol. Exp. Ther. **212:** 382–389, 1980.
74. DALY, J. W., PADGETT, W., CREVELING, C. R.; CANTACUZENE, D., AND KIRK, K. L.: Cyclic AMP-generating systems: regional differences in activation by adrenergic receptors in rat brain. J. Neurosci. **1:** 49–59, 1981.
75. DANOFF, S. K., AND YOUNG, J. M.: Is histamine potentiation of adenosine-stimulated cyclic AMP accumulation in guinea pig cerebral cortical slices mediated by products of inositol phospholipid breakdown? Biochem. Pharmacol. **36:** 1177–1179, 1987.
76. DAVIS, J. N., ARNETT, C. D., HOYLER, E., STALVEY, L. P., DALY, J. W., AND SKOLNICK, P. W.: Brain α-adrenergic receptors: comparison of ^{3}H-WB 4101 binding with norepinephrine stimulated cyclic AMP accumulation in rat cerebral cortex. Brain Res. **159:** 125–134, 1978.
77. DEBBAS, G., HOFFMAN, L., LANDON, E., AND HURWITZ, L.: Electron microscopic localization of calcium in vascular smooth muscle. Anat. Rec. **182:** 447–472, 1975.
78. DECKER, N., ERHARDT, J. D., LECLERC, G., AND SCHWARTZ, J.: Postjunctional α-adrenoceptors: α_1- and α_2-subtypes in rat vasculature in vitro and in vivo. Naunyn Schmiedebergs Arch. Pharmakol. **326:** 1–6, 1984.
79. DEHAYE, J.-P., HUGHES, B. P., BLACKMORE, P. F., AND EXTON, J. H.: Insulin inhibition of α-adrenergic actions in liver. Biochem. J. **194:** 949–956, 1981.
80. DEMARINIS, R. M., WISE, M., HIEBLE, J. P., AND RUFFOLO, R. R., JR.: Structure-activity relationships for alpha-1 adrenergic receptor agonists and antagonists. *In* The Alpha-1 Adrenergic Receptors, ed. by R. R. Ruffolo, Jr., pp. 211–265, Humana Press, Clifton, NJ, 1987.
81. DEMEY, J., AND VANHOUTTE, P. M.: Uneven distribution of postjunctional alpha-1 and alpha-2 like adrenoceptors in canine arterial and venous smooth muscle. Circ. Res. **48:** 875–884, 1981.
82. DESCOMBES, J-J., AND STOCLET, J-C.: Characterization of two distinct alpha-adrenoceptor binding sites in smooth muscle cell membranes from rat and bovine aorta. Naunyn Schmiedebergs Arch. Pharamkol. **329:** 282–288, 1985.
83. DETH, R. C., AND CASTEELS, R.: A study of releasable Ca fractions in smooth muscle cells of the rabbit aorta. J. Gen. Physiol. **69:** 401–416, 1977.
84. DETH, R. C., AND LYNCH,C. J.: Mobilization of a common source of smooth muscle Ca^{2+} by norepinephrine and methylxanthines. Am. J. Physiol. **240:** 239–247, 1981.
85. DETH, R., AND VAN BREEMEN, C.: Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release during drug induced activation of the rabbit aorta. Pfluegers Arch. Eur. J. Physiol. **348:** 13–23, 1974.
86. DETH, R., AND VAN BREEMEN, C.: Agonist induced release of intracellular calcium in the rabbit aorta. J. Membr. Biol. **30:** 363–380, 1977.
87. DEVINE, C. E., SOMLYO, A. V., AND SOMLYO, A. P.: Sarcoplasmic reticulum and excitation contraction coupling in mammalian smooth muscles. J. Cell Biol. **52:** 690–718, 1972.
88. DIGGES, K. G., AND SUMMERS, R. J.: Characterization of postsynaptic α-adrenoceptors in rat aortic strips and portal veins. Br. J. Pharmacol. **79:** 655–665, 1983.
89. DOCHERTY, J. R.: An investigation of presynaptic alpha-adrenoceptor subtypes in the pithed rat heart. Br. J. Pharmacol. **78:** 655–657, 1983.
90. DOCHERTY, J. R., AND MCGRATH, J. C.: A comparison of pre- and post-junctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat. Evidence for two types of post-junctional alpha-adrenoceptors. Naunyn-Schmiedebergs Arch. Pharmakol. **312:** 107–116, 1980.
91. DOGGRELL, S. A., AND PATON, D. M.: Effect of time and initial concentration of agonist on contractile responses in rat aorta. J. Pharmacol. Methods **1:** 115–119, 1978.
92. DOLPHIN, A. C., AND SCOTT, R. H.: Calcium channel currents and their inhibition by (−) baclofen in rat sensory neurones: modulation by guanine nucleotides. J. Physiol. **386:** 1–17, 1987.
93. DOWNES, C. P., HAWKINS, P. T., AND IRVINE, R. F.: Inositol 1,3,4,5-tetrakisphosphate and not phosphatidylinositol 3,4-bisphosphate is the probable precursor of inositol 1,3,4-trisphosphate in agonist-stimulated parotid glands. Biochem. J. **238:** 501–506, 1986.
94. DOWNING, O. A., WILSON, K. A., AND WILSON, V. G.: Competitive and noncompetitive blockade of α-adrenoceptors in rat aorta by prazosin. Br. J. Pharmacol. **73:** 290–291, 1981.
95. DREW, G. M.: What do antagonists tell us about a α-adrenoceptors? Clin. Sci. **68** (suppl. 10): 15s–19s, 1985.
96. DREW, G. M., AND WHITING, S. B.: Evidence for two distinct types of postsynaptic α-adrenoceptor in vascular smooth muscle in vivo. Br. J. Pharmacol. **67:** 207–215, 1979.
97. DROOGMANS, G., RAEYMAEKERS, L., AND CASTEELS, R.: Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. J. Gen. Physiol. **70:** 129–148, 1977.
98. DUMAN, R. S., KARBON, E. W., HARRINGTON, C., AND ENNA, S. J.: An examination of the involvement of phospholipases A_2 and C in the α-adrenergic and γ-aminobutyric acid receptor modulation of cyclic AMP accumulation in rat brain slices. J. Neurochem. **47:** 800–810, 1986.
99. DUMAN, R. S., STRADA, S. J., AND ENNA, S. J.: Effect of imipramine and adrenocorticotropin administration on the rat brain norepinephrine-coupled cyclic nucleotide generating systems: alterations in alpha and beta components. J. Pharmacol. Exp. Ther. **234:** 409–414, 1985.
100. EDMAN, K. A. P., AND SCHILD, H. O.: Calcium and the stimulant and inhibitory effects of adrenaline in depolarized smooth muscle. J. Physiol. **169:** 404–411, 1963.
101. ENDO, M.: Calcium release from the sarcoplasmic reticulum. Physiol. Rev. **57:** 71–108, 1977.
102. FAIN, J. N., AND BERRIDGE, M. J.: Relationship between hormonal activation of phosphatidylinositol hydrolysis, fluid secretion, and calcium flux in the blowfly salivary gland. Biochem. J. **178:** 45–58, 1979.
103. FAIN, J. N., AND GARCIA-SAINZ, J. A.: Role of phosphatidylinositol turnover in α_1- and adenylate cyclase inhibition in α_2-effects of catecholamines. Life Sci. **26:** 1183–1194, 1980.
104. FLAVAHAN, N. A., AND MCGRATH, J. C.: α_1-Adrenoceptors can mediate chronotropic responses in the rat heart. Br. J. Pharmacol **73:** 586–588, 1981.
105. FLAVAHAN, N. A., AND VANHOUTTE, P. M.: α_1-Adrenoceptor subclassification in vascular smooth muscle. Trends Pharmacol. Sci. **7:** 347–349, 1986.
106. FLECKENSTEIN, A.: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Annu. Rev. Pharmacol. Toxicol. **17:** 149–166, 1977.
107. FOWLER, P. J., GROUS, M., PRICE, W., AND MATTHEWS, W. D.: Pharmacological differentiation of postsynaptic alpha-adrenoceptors in the dog saphenous vein. J. Pharmacol. Exp. Ther. **229:** 712–718, 1984.
108. FOX, A. P., NOWYCKY, M. C., AND TSIEN, R. W.: Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. J. Physiol. **394:** 149–172, 1987.
109. FOX, A. P., NOWYCKY, M. C., AND TSIEN, R. W.: Single-channel recordings of three types of calcium channels in chick sensory neurones. J. Physiol. **394:** 173–200, 1987.
110. FOX, A. W., ABEL, P. W., AND MINNEMAN, K. P.: Activation of α_1-adrenoceptors increases ^{3}H-inositol metabolism in rat vas deferens and caudal artery. *Eur. J. Pharmacol.* **116:** 145–152, 1985.
111. FRIEDMAN, M. E., SUAREZ-KURTZ, G., KACZOROWSKI, G. J., KATZ, G. M., AND REUBEN, J. P.: Two calcium currents in a smooth muscle cell line. Am. J. Physiol. **250:** H699–H703, 1986.
112. FURCHGOTT, R. F., AND BURSTZYN, P.: Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors. Ann. NY Acad. Sci. **139:** 882–899, 1967.
113. GARCIA-SAINZ, J. A., AND HERNANDEZ-SOTOMAYOR, S. M. T.: Adrenergic regulation of gluconeogenesis: possible involvement of two mechanisms of signal transduction in α_1-adrenergic action. Proc. Natl. Acad. Sci. USA **82:** 6727–6730, 1985.
114. GILMAN, A. G.: G proteins and dual control of adenylate cyclase. Cell **36:** 577–579, 1984.
115. GODFRAIND, T.: Mechanisms of action of calcium entry blockers. Fed. Proc. **40:** 2866–2871, 1981.
116. GODFRAIND, T., AND MILLER, R. C.: Specificity of action of Ca^{2+} entry blockers: comparison of their actions in rat arteries and in human coronary arteries. Circ. Res. **52** (suppl. 1): I81–I91, 1983.
117. GODFRAIND, T., MILLER, R. C., AND LIMA, J. S.: Selective α_1- and α_2-adrenoceptor agonist induced contractions and Ca fluxes in the rat isolated aorta. Br. J. Pharmacol. **77:** 597–604, 1982.
118. GOLENHOFEN, K., AND HERMSTEIN, N.: Differentiation of calcium activation mechanisms in vascular smooth muscle by selective suppression with verapamil and D600. Blood Vessels **12:** 21–37, 1975.
119. GOMPERTS, B. D.: Involvement of guanine nucleotide binding protein in the gating of calcium by receptors. Nature (Lond.) **306:** 64–66, 1983.
120. GONZALEZ, R. A., AND CREWS, F. T.: Cholinergic and adrenergic stimulated inositide hydrolysis in brain: interaction, regional distribution, and coupling mechanism. J. Neurochem. **45:** 1076–1084, 1985.
121. GONZALEZ, R. A., AND CREWS, F. T.: Guanine nucleotides stimulate production of inositol trisphosphate in rat cortical membranes. Biochem. J. **232:** 799–804, 1985.
122. GONZALEZ, R. A., FELDSTEIN, J. B., CREWS, F. T., AND RAIZADA, M. K.: Receptor-mediated inositide hydrolysis is a neuronal response: comparison of primary neuronal and glial cultures. Brain Res **345:** 350–355, 1985.
123. GOODHART, M., FERRY, N., GEYNET, P., AND HANOUNE, J.: Hepatic α_1-

adrenergic receptors show agonist-specific regulation by guanine nucleotides. J. Biol. Chem. **257:** 11577–11583, 1982.
124. GUSOVSKY, F., HOLLINGSWORTH, E. B., AND DALY, J. W.: Regulation of phosphatidylinositol turnover in brain synaptoneurosomes: stimulatory effects of agents that enhance influx of sodium ions. Proc. Natl. Acad. Sci. USA **83:** 3003–3007, 1986.
125. HAEUSLER, G.: Differential effect of verapamil on excitation-contraction coupling in smooth muscle and on excitation-secretion coupling in adrenergic nerve terminals. J. Pharm. Exp. Ther. **180:** 672–682, 1972.
126. HAEUSLER, G., RICHARDS, J. G., AND THORENS, S.: Noradrenaline contractions in rabbit mesenteric arteries skinned with saponin. J. Physiol. **321:** 537–556, 1981.
127. HALLCHER, L. J., AND SHERMAN, W. R.: The effects of lithium ion and other agents on the activity of myo-inositol-1-phosphatase from bovine brain. J. Biol. Chem. **255:** 10896–10901, 1980.
128. HAMED, A. T., JOHNSON, T. D., CHARLTON, K. G., AND CLARKE, D. E.: Pharmacological characterization of α-adrenoceptor subtypes in rat isolated thoracic aorta. J. Auton. Pharmacol. **3:** 265–273, 1983.
129. HAMILTON, C. A., REID, J. L., AND SUMNER, D. J.: Acute effects of phenoxybenzamine on α-adrenoceptor responses in vivo and in vitro: relation of in vivo pressor responses to the number of specific adrenoceptor binding sites. J. Cardiovasc. Pharmacol. **5:** 868–873, 1983.
130. HAN, C., ABEL, P. W., AND MINNEMAN, K. P.: Heterogeneity of α_1-adrenergic receptors revealed by chlorethylclonidine. Mol. Pharmacol. **32:** 505–510, 1987.
131. HAN, C., ABEL, P. W., AND MINNEMAN, K. P.: α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature (Lond.) **329:** 333–335, 1987.
132. HARDEN, T. K., STEPHENS, L., HAWKINS, P. T., AND DOWNES, C. P.: Turkey erythrocyte membranes as a model for regulation of phospholipase C by guanine nucleotides. J. Biol. Chem. **262:** 9057–9061, 1987.
133. HAUSER, G., AND SMITH, T. L.: Characteristics of the norepinephrine-stimulated phosphatidylinositol turnover in rat pineal cell dispersions. Neurochem. Res. **6:** 1067–1079, 1981.
134. HEPLER, J. R., AND HARDEN, T. K.: Guanine nucleotide-dependent pertussis-toxin-insensitive stimulation of inositol phosphate formation by carbachol in a membrane preparation from human astrocytoma cells. Biochem. J. **239:** 141–146, 1986.
135. HERMSMEYER, K.: Contraction and membrane activation in several mammalian vascular muscles. Life Sci. **10:** 223–234, 1971.
136. HESCHELER, J., ROSENTHAL, W., TRAUTWEIN, W., AND SCHULTZ, G.: The GTP-binding protein, G_o, regulates neuronal calcium channels. Nature (Lond.) **325:** 445–447, 1987.
137. HESLOP, J. P., IRVINE, R. F., TASHIJAN, A. H., JR., AND BERRIDGE, M. J.: Inositol tetrakis- and pentakisphosphates in GH_4 cells. J. Exp. Biol. **119:** 395–401, 1985.
138. HIEBLE, J. P., DEMARINIS, R. M., AND MATTHEWS, W. D.: Evidence for and against heterogeneity of alpha-1 adrenoceptors. Life Sci **38:** 1339–1350, 1986.
139. HIEBLE, J. P., MATTHEWS, W. D., DEMARINIS, R. M., AND RUFFOLO, R. R., JR.: Heterogeneity of alpha-1 adrenergic receptors. *In* The Alpha-1 Adrenergic Receptors, ed. by R. R. Ruffolo, Jr., pp. 325–349, Humana Press, Clifton, NJ, 1987.
140. HIEBLE, J. P., SARAU, H. M., FOLEY, J. J., DEMARINIS, R. M., AND PENDLETON, R. G.: Comparison of central and peripheral alpha-1-adrenoceptors. Naunyn Schmiedebergs Arch. Pharmakol. **318:** 267–273, 1982.
141. HIEBLE, J. P., AND WOODWARD, D. F.: Different characteristics of postjunctional alpha-adrenoceptors on arterial and venous smooth muscle. Naunyn Schmiedebergs Arch. Pharmakol. **318:** 44–50, 1984.
142. HO, A., AND KLEIN, D. C.: Activation of α_1-adrenoceptors, protein kinase C, or treatment with intracellular free Ca^{2+} elevating agents increases pineal phospholipase A_2 activity: evidence that protein kinase C may participate in Ca^{2+} dependent α_1-adrenergic stimulation of pineal phospholipase A_2 activity. J. Biol. Chem. **262:** 11764–11770, 1987.
143. HOFFMAN, B. B.: Adrenergic receptor activating drugs and adrenergic receptor blocking drugs. *In* Basic and Clinical Pharmacology, ed. by B. G. Katzung, pp. 86–106, Lange Medical Publications, Los Altos, CA, 1984.
144. HOFFMAN, S. L., AND MAJERUS, P. W.: Identification and properties of two distinct phosphatidylinositol-specific phospholipase C enzymes from sheep seminal vesicular glands. J. Biol. Chem. **257:** 6461–6469, 1982.
145. HOKIN, L. E., AND SHERWIN, A. L.: Protein secretion and phosphate turnover in the phospholipids in salivary glands in vitro. J. Physiol. **135:** 18–29, 1957.
146. HOLCK, M., AND GEROLD, M.: Calcium entry blockers inhibit vasoconstrictor responses to sympathetic nerve stimulation mediated by α_1-adrenoceptors. Naunyn Schmiedebergs Arch. Pharmakol. **330:** 22–32, 1985.
147. HOLCK, M. I., JONES, C. H. M., AND HAEUSLER, G.: Differential interactions of clonidine and methoxamine with the postsynaptic α-adrenoceptor of rabbit main pulmonary artery. J. Cardiovasc. Pharmacol. **5:** 240–248, 1983.
148. HOLLINGSWORTH, E. B., SEARS, E. B., AND DALY, J. W.: An activator of protein kinase C (phorbol-12-myristate-13-acetate) augments 2-chloroadenosine elicited accumulation of cyclic AMP in guinea pig cerebral cortical particulate preparations. FEBS Lett. **184:** 339–342, 1985.
149. HOLZ, G. G., RANE, S. G., AND DUNLAP, K.: GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. Nature (Lond.) **319:** 670–672, 1986.
150. HORN, A. S., AND PHILLIPSON, O. T.: A noradrenaline sensitive adenylate cyclase in the rat limbic forebrain: preparation, properties, and the effects of agonists, adrenolytics, and neuroleptic drugs. Eur. J. Pharmacol. **37:** 1–11, 1976.
151. HOYER, D.: Characterization of IBE 2254 binding to alpha adrenergic receptors on intact DDT1 smooth muscle cells: comparison with membrane binding and correlation with phosphoinositides breakdown. J. Recept. Res. **4:** 51–67, 1984.
152. HUDGINS, P., AND WEISS, G. B.: Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine, and potassium. J. Pharmacol. Exp. Ther. **159:** 91–97, 1968.
153. HURWITZ, L., AND SURIA, A.: The link between agonist action and response in smooth muscle. Annu. Rev. Pharmacol. **11:** 303–326, 1971.
154. HUTSON, N. J., BRUMLEY, F. T., ASSIMACOPOULOS, F. D., HARPER, S. C., AND EXTON, J. H.: Studies on the α-adrenergic activation of hepatic glucose output. I. Studies on the α-adrenergic activation of phosphorylase and gluconeogenesis and inactivation of glycogen synthase in isolated rat liver parenchymal cells. J. Biol. Chem. **251:** 5200–5208, 1976.
155. IRVINE, R. F., LETCHER, A. J., HESLOP, J. R., AND BERRIDGE, M. J.: The inositol tris/tetrakisphosphate pathway—demonstration of Ins(1,4,5)P_3 kinase activity in animal tissues. Nature (Lond.) **320:** 631–634, 1986.
156. IRVINE, R. F., AND MOOR, R. M.: Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external calcium. Biochem. J. **240:** 917–920, 1986.
157. ISMAIL, S., JAHN, U., AND WEETMAN, D. F.: Sgd 101/75 (4(2-imidazolineamino)-2-methylindazol-chlorhydrate): a drug that can act as an agonist, partial agonist, or antagonist on α-adrenoceptors of isolated tissues. Br. J. Pharmacol. **72:** 535–536P, 1981.
158. ISOM, L. L., CRAGOE, E. J., AND LIMBIRD, L. E.: α_2-Adrenergic receptors accelerate Na^+/H^+ exchange in neuroblastoma × glioma cells. J. Biol. Chem. **262:** 6750–6757, 1987.
159. ITOH, T., KURIYAMA, H., AND SUZUKI, H.: Differences and similarities in the noradrenaline and caffeine induced mechanical responses in the rabbit mesenteric artery. J. Physiol. **337:** 609–629, 1983.
160. JAGADEESH, G., AND DETH, R. C.: Different affinity states of alpha-1 adrenergic receptors defined by agonists and antagonists in bovine aorta plasma membrane. J. Pharmacol. Exp. Ther. **243:** 430–436, 1987.
161. JAMES, M. K., AND LEIGHTON, H. J.: Analysis of putative alpha-1s adrenoceptor agonism by Sgd 101/75 in the rat anococcygeus muscle. J. Pharmacol. Exp. Ther. **242:** 772–776, 1987.
162. JANIS, R. A., AND TRIGGLE, D. J.: New developments in Ca^{2+} channel antagonists. J. Med. Chem. **26:** 775–785, 1983.
163. JENKINSON, D. H., AND MORTON, I. K. M.: The effect of noradrenaline on the permeability of depolarized intestinal smooth muscle to inorganic ions. J. Physiol. **188:** 373–386, 1967.
164. JIM, K. F., DEMARINIS, R. M., AND MATTHEWS, W. D.: Measurements of $^{45}Ca^{2+}$ uptake and contractile responses after activation of postsynaptic α_1-adrenoceptors in the isolated canine saphenous vein: effects of calcium entry blockade. Eur. J. Pharmacol. **107:** 199–208, 1985.
165. JIM, K. F., MACIA, R. A., AND MATTHEWS, W. D.: An evaluation of the ability of a series of full alpha-1 adrenoceptor agonists to release internal calcium in venous smooth muscle. J. Pharmacol. Exp. Ther. **235:** 377–381, 1985.
166. JIM, K. F., MACIA, R. A., AND MATTHEWS, W. D.: Role of receptor reserve in the inhibition of alpha-1 adrenoceptor mediated pressor responses by calcium antagonists in the pithed rat. J. Pharmacol. Exp. Ther. **238:** 89–94, 1986.
167. JOHNSON, R. D., AND MINNEMAN, K. P.: α_1-Adrenergic receptors and stimulation of ^{3}H-inositol metabolism in rat brain: regional distribution and parallel inactivation. Brain Res. **341:** 7–15, 1985.
168. JOHNSON, R. D., AND MINNEMAN, K. P.: Characterization of α_1-adrenoceptors which increase cyclic AMP accumulation in rat cerebral cortex. Eur. J. Pharmacol. **129:** 293–305, 1986.
169. JOHNSON, R. D., AND MINNEMAN, K. P.: Differentiation of α_1-adrenergic receptors linked to phosphatidylinositol turnover and cyclic AMP accumulation in rat brain. Mol. Pharmacol. **31:** 239–246, 1987.
170. JONES, D. J.: Adenosine regulation of cyclic 3′5′-adenosine monophosphate formation in rat spinal cord. J. Pharmacol. Exp. Ther. **219:** 370–376, 1981.
171. JONES, D. J., AND MCKENNA, L. F.: Alpha adrenergic receptor mediated formation of cyclic AMP in rat spinal cord. J. Cyclic Nucleotide Res. **6:** 133–141, 1980.
172. JONES, L. M., AND MICHELL, R. H.: Breakdown of phosphatidylinositol provoked by muscarinic cholinergic stimulation of rat parotid gland fragments. Biochem. J. **142:** 583–590, 1974.
173. JONES, S. B., SMITH, J. M., JONES, A. W., AND BYLUND, D. B.: Alpha-1 adrenergic receptor binding in aortas from rat and dog: comparison of ^{3}H-prazosin and β-iodo(^{125}I)I)-4-hydroxyphenyl-ethyl-aminomethyl-tetralone. J. Pharmacol. Exp. Ther. **241:** 875–881, 1987.
174. JOSEPH, S. K., THOMAS, A. P., WILLIAMS, R. J., IRVINE, R. F., AND WILLIAMSON, J. R.: Myo-inositol-1,4,5-trisphosphate. A second messenger for the hormonal mobilization of intracellular calcium in liver. J. Biol. Chem. **259:** 3077–3081, 1984.

175. JOSEPH, S. K., AND WILLIAMSON, J. R.: The origin, quantitation, and kinetics of intracellular calcium mobilization by vasopressin and phenylephrine in hepatocytes. J. Biol. Chem. **258:** 10425–10432, 1983.
176. KARAKI, H., KUBOTA, H., AND URAKAWA, N.: Mobilization of stored calcium for phasic contraction induced by norephinephrine in rabbit aorta. Eur. J. Pharmacol. **56:** 237–245, 1979.
177. KARAKI, H., AND WEISS, G. B.: Calcium release in smooth muscle. Life Sci. **42:** 111–122, 1988.
178. KARLINER, J. S., BARNES, P., HAMILTON, C. A., AND DOLLERY, C. T.: Alpha-1 adrenergic receptors in guinea pig myocardium: identification by binding of a new radioligand ^{3}H-prazosin. Biochem. Biophys. Res. Commun. **90:** 142–149, 1979.
179. KASS, R. S., AND SANGUINETTI, M. C.: Calcium channel inactivation in the calf cardiac Purkinje fiber: evidence for voltage- and calcium-mediated mechanisms. J. Gen. Physiol. **84:** 705–726, 1984.
180. KAWAHARA, R. S., AND BYLUND, D. B.: Solubilization and characterization of putative α_2-adrenergic isoreceptors from the human platelet and the rat cerebral cortex. J. Pharmacol. Exp. Ther. **233:** 603–610, 1985.
181. KENAKIN, T. P.: The relative contribution of affinity and efficacy to agonist activity: organ selectivity of noradrenaline and oxymetazoline with reference to the classification of drug receptors. Br. J. Pharmacol. **81:** 131–141, 1984.
182. KENAKIN, T. P.: The classification of drugs and drug receptors in isolated tissues. Pharmacol. Rev. **36:** 165–222, 1984.
183. KENDALL, D. A., DUMAN, R., SLOPIS, J., AND ENNA, S. J.: Influence of adrenocorticotropic hormone and yohimbine on antidepressant-induced declines in rat brain neurotransmitter receptor binding and function. J. Pharmacol. Exp. Ther. **222:** 566–571, 1982.
184. KENDALL, D. A., AND NAHORSKI, S. R.: Inositol phospholipid hydrolysis in rat cerebral cortical slices. II. Calcium requirement. J. Neurochem. **42:** 1384–1394, 1984.
185. KENDALL, D. A., AND NAHORSKI, S. R.: Dihydropyridine calcium channel activators and antagonists influence depolarisation-evoked inositol phospholipid hydrolysis in brain. Eur. J. Pharmacol. **115:** 31–36, 1985.
186. KLEIN, D. C., SUGDEN, D., AND WELLER, J. L.: Postsynaptic α_1-adrenergic receptors potentiate the β-adrenergic stimulation of pineal serotonin N-acetyltransferase. Proc. Natl. Acad. Sci. USA **80:** 599–603, 1983.
187. KNEER, N. M., AND LARDY, H. A.: Regulation of gluconeogenesis by norepinephrine, vasopressin, and angiotensin II: A comparative study in the absence and presence of extracellular calcium. Arch. Biochem. Biophys. **225:** 187–195, 1983.
188. KNIEPP, S. M., AND RUTLEDGE, C. O.: Effects of calcium depletion on norepinephrine and A23187-induced stimulation of inositol phosphate formation. Biochem. Pharmacol. **36:** 3043–3050, 1987.
189. KOBILKA, B. K., MATSUI, H., KOBILKA, T. S., YANG-FENG, T. L., FRANCKE, U., CARON, M. G., LEFKOWITZ, R. J., AND REGAN, J. W.: Cloning, sequencing, and expression of the gene coding for the human platelet α_2-adrenergic receptor. Science (Wash. DC) **238:** 650–656, 1987.
190. KOBINGER, W., AND PICHLER, L.: Presynaptic activity of the imidazolidine derivative ST 587, a highly selective α_1-adrenoceptor agonist. Eur. J. Pharmacol. **82:** 203–206, 1982.
191. KORSTANJE, C., WILFFERT, B., DE JONGE, A., THOOLEN, M. J. M. C., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A. Calcium influx-dependent and -independent α_1-adrenoceptor-mediated processes of vasoconstriction do not operate via different α_1-adrenoceptor subtypes. J. Cardiovasc. Pharmacol. **6:** 1102–1108, 1984.
192. KOWARSKI, D., SHUMAN, H., SOMLYO, A. P., AND SOMLYO, A. V.: Calcium release by noradrenaline from central sarcoplasmic reticulum in rabbit main pulmonary artery smooth muscle. J. Physiol. **366:** 153–175, 1985.
193. KUNO, M., AND GARDNER, P.: Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. Nature (Lond.) **326:** 301–304, 1987.
194. KUNO, M., GORONZY, J., WEYAND, C. M., AND GARDNER, P.: Single-channel and whole-cell recordings of mitogen-regulated inward currents in cloned helper T-lymphocytes. Nature (Lond.) **323:** 269–273, 1986.
195. LANDS, A. M., ARNOLD, A., MCAULIFF, J. P., LUDUENA, F. P., AND BROWN, T. G.: Differentiation of receptor systems activated by sympathomimetic amines. Nature (Lond.) **214:** 597–598, 1967.
196. LANDS, A. M., LUDUENA, F. P., AND BUZZO, H. J.: Differentiation of receptors responsive to isoproterenol. Life Sci. **8:** 373–382, 1967.
197. LANGER, S. Z.: Presynaptic regulation of catecholamine release. Br. J. Pharmacol. **60:** 481–497, 1974.
198. LANGER, S. Z., AND HICKS, P. E.: Alpha-adrenoceptor subtypes in blood vessels: physiology and pharmacology. J. Cardiovasc. Pharmacol. **66:** 547s–556s, 1984.
199. LANGER, S. Z., MASSINGHAM, R., AND SHEPPERSON, N. B.: Presence of postsynaptic α_2-adrenoceptors of predominantly extrasynaptic location in the vascular smooth muscle of the dog hind limb. Clin. Sci. **59:** 225s–228s, 1980.
200. LANGER, S. Z., AND SHEPPERSON, N. B.: Recent developments in vascular smooth muscle pharmacology: the postsynaptic α_2-adrenoceptor. Trends Pharmacol. Sci. **3:** 440–444, 1982.
201. LEBLANC, G. G., AND CIARANELLO, R. D.: α-Noradrenergic potentiation of neurotransmitter-stimulated cAMP production in rat striatal slices. Brain Res. **293:** 57–65, 1984.
202. LECLERC, G., ROUOT, G., SCHWARTZ, J., VELLY, J., AND WEMUTH, C. G.: Studies on some para-substituted clonidine derivatives that exhibit an α-adrenoceptor stimulant activity. Br. J. Pharmacol. **71:** 5–9, 1980.
203. LELTEN, P., CAUVIN, C., LODGE, N., SAIDA, K., AND VAN BREEMEN, C.: Ca^{2+} sources mobilized by α_1-receptor activation in vascular smooth muscle. Clin. Sci. **68** (suppl. 10): 47s–50s, 1985.
204. LELTEN, P. A. A., AND VAN BREEMEN, C. The effects of caffeine on the noradrenaline sensitive calcium store in rabbit aorta. J. Physiol. **357:** 327–339, 1984.
205. LEW, M. J., AND ANGUS, J. A.: α_1- and α_2-adrenoceptor mediated pressor responses: are they differentiated by calcium antagonists or by functional antagonism? J. Cardiovasc. Pharmacol. **7:** 401–408, 1985.
206. LEWIS, D. L., WEIGHT, F. F., AND LUINI, A.: A guanine nucleotide binding protein mediates the inhibition of voltage-dependent calcium current by somatostatin in a pituitary cell line. Proc. Natl. Acad. Sci. USA **83:** 9035–9039, 1986.
207. LIMBIRD, L. E.: Activation and attenuation of adenylate cyclase: the role of GTP-binding proteins as macromolecular messengers in receptor-cyclase coupling. Biochem. J. **195:** 1–13, 1981.
208. LIPPTON, H. L., ARMSTEAD, W. M., HYMAN, A. L., AND KADOWITZ, P. J.: Influence of calcium entry blockade on vasoconstrictor responses in feline mesenteric vascular bed. Circ. Res. **61:** 570–580, 1987.
209. LITOSCH, I., AND FAIN, J. N.: Regulation of phosphoinositide breakdown by guanine nucleotides. Life Sci. **39:** 187–194, 1986.
210. LITOSCH, I., WALLACE, C., AND FAIN, J. N.: 5-Hydroxytryptamine stimulates inositol phosphate production in a cell-free system from blowfly salivary glands. J. Biol. Chem. **260:** 5464–5471, 1985.
211. LJUNG, B., AND KJELLSTEDT, A.: Functional antagonism of noradrenaline responses by felodipine and other calcium antagonists in vascular smooth muscles. J. Cardiovasc. Pharmacol. **10:** (suppl. 1): 82s–88s, 1987.
212. LLENAS, J., AND MASSINGHAM, R.: A study of compounds which inhibit vascular smooth muscle contraction. Eur. J. Pharmacol. **22:** 75–82, 1983.
213. LLINAS, R., AND SUGIMORI, M.: Electrophysiological properties of in vitro Purkinje cell somata in mammalian cerebellar slices. J. Physiol. **305:** 171–195, 1980.
214. LYNCH, C. J., BLACKMORE, P. F., CHAREST, R., AND EXTON, J. H.: The relationship between receptor binding capacity for norepinephrine, angiotensin II, and vasopressin and release of inositol trisphosphate, calcium mobilization, and phosphorylase activation in rat liver. Mol. Pharmacol. **28:** 93–99, 1985.
215. LYNCH, C. J., CHAREST, R., BLACKMORE, P. F., AND EXTON, J. H.: Studies on the hepatic α_1-adrenergic receptors: modulation of guanine nucleotide effects by calcium, temperature, and age. J. Biol. Chem. **260:** 1593–1600, 1984.
216. MACIA, R. A., MATTHEWS, W. D., LAFFERTY, J., AND DEMARINIS, R. M.: Assessment of alpha-adrenergic receptor subtypes in isolated rat aortic segments. Naunyn Schmiedebergs Arch. Pharmakol. **325:** 306–309, 1984.
217. MAGARIBUCHI, T., AND KURIYAMA, H.: Effects of noradrenaline and isoprenaline on the electrical and mechanical activities of guinea-pig depolarized taenia coli. Jpn. J. Physiol. **22:** 253–270, 1972.
218. MAGISTRETTI, P. J., AND SCHORDERET, M.: VIP and noradrenaline act synergistically to increase cyclic AMP in cerebral cortex. Nature (Lond.) **308:** 280–282, 1984.
219. MAGISTRETTI, P. J., AND SCHORDERET, M.: Norepinephrine and histamine potentiate the increases in cyclic adenosine 3′5′-monophosphate elicited by vasoactive intestinal polypeptide in mouse cerebral cortical slices: mediation by α_1-adrenergic and H_1-histaminergic receptors. J. Neurosci. **5:** 362–368, 1985.
220. MAIER, K. U., AND RUTLEDGE, C. O.: Comparison of norepinephrine and veratrine induced phosphoinositide hydrolysis in rat brain. J. Pharmacol. Exp. Ther. **240:** 729–736, 1987.
221. MAJERUS, P. W., CONNOLLY, T. M., DECKMYN, H., ROSS, T. S., BROSS, T. E., ISHII, H., BANSAL, V. S., AND WILSON, D. B.: The metabolism of phosphoinositide-derived messenger molecules. Science (Wash. DC) **234:** 1519–1526, 1986.
222. MATTHEWS, W. D., MACIA, R. A., BECKERINGH, J. J., DEMARINIS, R. M., DE JONGE, A., THOOLEN, M. J. M. C., WILFFERT, B., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Calcium utilization in the vasoconstriction to enantiomers of SK&F 89748-A. J. Pharmacol. Exp. Ther. **232:** 330–336, 1985.
223. MCCLESKEY, E. W., FOX, A. P., FELDMAN, D., CRUZ, L. J., OLIVERA, B. M., TSIEN, R. W., AND YOSHIKAMI, D.: Omega-conotoxin: direct and persistent blockade of specific types of calcium channels in neurons but not muscle. Proc. Natl. Acad. Sci. USA **84:** 4327–4331, 1987.
224. MCDONALD, A., AND MCGRATH, J. C.: The distribution of adrenoceptors and other drug receptors between the two ends of the rat vas deferens as revealed by selective agonists and antagonists. Br. J. Pharmacol. **71:** 445–458, 1980.
225. MCDONOUGH, P. M., WETZEL, G. T., AND BROWN, J. H.: Further characterization of the presynaptic alpha-1 receptor modulating ^{3}H ACh release from rat atria. J. Pharmacol. Exp. Ther. **238:** 612–617, 1986.
226. MCGRATH, J. C.: Adrenergic and "non-adrenergic" components in the contractile response of the vas deferens to a single indirect stimulus. J. Physiol. **283:** 23–39, 1978.

227. McGRATH, J. C.: Evidence for more than one type of postjunctional alpha-adrenoceptor. Biochem. Pharmacol. **31:** 467–484, 1982.
228. McGRATH, J. C.: Ca^{2+}-free and Ca^{2+} requiring response in rat anococcygeus muscle have differing agonist potency series. Br. J. Pharmacol. **79:** 227p, 1983.
229. McGRATH, J. C.: α-Adrenoceptor antagoniam by apoyohimbine and some obeservations on the pharmacology of α-adrenoceptors in the rat anococcygeus and vas deferens. Br. J. Pharmacol. **82:** 769–781, 1984.
230. McGRATH, J. C.: α-Adrenoceptor agonists and the Ca^{2+} dependence of smooth muscle contraction: evidence for subtypes of receptors or for agonist-dependent differences in the agonist-receptor interaction? Clin. Sci. **68** (suppl. 10): 55s–63s, 1985.
231. McPHERSON, G. A., AND SUMMERS, R. J.: ^{3}H-Prazosin and ^{3}H-clonidine binding to α-adrenoceptors in membranes prepared from regions of rat kidney. Br. J. Pharmacol. **77:** 177–184, 1982.
232. MEDGETT, I. C., AND LANGER, S. Z.: Heterogeneity of smooth muscle alpha-1 adrenoceptors in rat tail artery in vitro. J. Pharmacol. Exp. Ther. **229:** 823–830, 1984.
233. MEISHERI, K. D., HWANG, O., AND VAN BREEMEN, C.: Evidence for two separate Ca^{2+} pathways in smooth muscle plasmalemma. J. Membr. Biol. **59:** 19–25, 1981.
234. MELDOLESI. J., AND POZZAN, T.: Pathways of Ca^{2+} influx at the plasma membrane: voltage-, receptor-, and second messenger-operated channels. Exp. Cell Res. **171:** 271–283, 1987.
234a. MERRITT, J. E., AND RINK, T. J.: The effects of substance P and carbachol on inositol tris- and tetrakis-phosphate formation and cytosolic free calcium in rat parotid acinar cells: a correlation between inositol phosphate levels and calcium entry. J. Biol. Chem. **262:** 14912-14916, 1987.
235. MERRITT, J. E., TAYLOR, C. W., RUBIN, R. P., AND PUTNEY, J. W.: Isomers of inositol trisphosphate in exocrine pancreas. Biochem. J. **238:** 825–829, 1986.
236. MERRITT, J. E., TAYLOR, C. W., RUBIN, R. P., AND PUTNEY, J. W.: Evidence suggesting that a novel guanine nucleotide regulatory protein couples receptors to phospholipase C in exocrine pancreas. Biochem. J. **236:** 337–343, 1986.
237. MICHELL, R. H.: Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta **415:** 81–147, 1975.
238. MICHELL, R. H., KIRK, C. J., JONES, L. M., DOWNES, C. P., AND CREBA, J. A.: The stimulation of inositol lipid metabolism that accompanies calcium mobilization in stimulated cells: defined characteristics and unanswered questions. Philos. Trans. R. Soc. Lond. B Sci. **296:** 123–137, 1981.
239. MINNEMAN, K. P.: Binding properties of alpha-1 adrenergic receptors in rat cerebral cortex: similarlity to smooth muscle. J. Pharmacol. Exp. Ther. **227:** 605–612, 1983.
240. MINNEMAN, K. P.: Phenoxybenzamine is more potent in inactivating α_1- than α_2-adrenergic receptor binding sites. Eur. J. Pharmacol. **94:** 171–174, 1983.
241. MINNEMAN, K. P., AND ABEL, P. W.: "Spare" α_1-adrenergic receptors and the potency of agonists in rat vas deferens. Mol. Pharmacol. **25:** 56–63, 1984.
242. MINNEMAN, K. P., AND ABEL, P. W.: Relationship between α_1-adrenoceptor density and functional response of rat vas deferens. Studies with phenoxybenzamine. Naunyn Schmiedebergs Arch. Pharmakol. **327:** 238–246, 1984.
243. MINNEMAN, K. P., AND ABEL, P. W.: Alpha-1-adrenergic receptor binding and contraction of rat caudal artery. J. Pharmacol. Exp. Ther. **239:** 678–686, 1986.
244. MINNEMAN, K. P., AND ABEL, P. W.: Relationship of alpha-1 adrenergic receptor occupancy to tissue response. *In* The Alpha-1 Adrenergic Receptors, ed. by R. R. Ruffolo, Jr., pp. 267–324, Humana Press, Clifton, NJ, 1987.
245. MINNEMAN, K. P., FOX, A. W., AND ABEL, P. W.: Occupancy of alpha-1 adrenergic receptors and contraction of rat vas deferens. Mol. Pharmacol. **22:** 359–368, 1983.
246. MINNEMAN, K. P., HAN, C., AND ABEL, P. W.: Comparison of α_1-adrenergic receptor subtypes distinguished by chlorethylclonidine and WB 4101. Mol. Pharmacol. **33:** 509–514, 1988.
247. MINNEMAN, K. P., AND JOHNSON, R. D.: Characterization of alpha-1 adrenergic receptors linked to ^{3}H-inositol metabolism in rat cerebral cortex. J. Pharmacol. Exp. Ther. **230:** 317–323, 1984.
248. MINNEMAN, K. P., PITTMAN, R. N., AND MOLINOFF, P. B.: β-Adrenergic receptor subtypes: properties, distribution, and regulation. Annu. Rev. Neurosci **4:** 419–461, 1981.
249. MOBLEY, P. L., MANIER, D. H., AND SULSER, F.: Norepinephrine-sensitive adenylate cyclase system in rat brain: role of adrenal corticosteroids. J. Pharmacol. Exp. Ther. **226:** 71–77, 1983.
250. MOBLEY, P. L., AND SULSER, F.: Adrenal corticoids regulate sensitivity of noradrenaline receptor coupled adenylate cyclase in brain. Nature (Lond.) **286:** 608–609, 1980.
251. MORGAN, N. G., BLACKMORE, P. F., AND EXTON, J. H.: Age-related changes in the control of hepatic cyclic AMP levels by α_1- and β_2-adrenergic receptors in male rats. J. Biol. Chem. **258:** 5103–5109, 1983.
252. MORGAN, N. G., WAYNICK, L. E., AND EXTON, J. H.: Characterisation of the α_1-adrenergic control of hepatic cAMP in male rats. Eur. J. Pharmacol. **96:** 1-10, 1983.
253. MORROW, A. L., BATTAGLIA, G., NORMAN, A. B., AND CREESE, I.: Identification of subtypes of ^{3}H-prazosin labeled α_1-receptor binding sites in rat brain. Eur. J. Pharmacol. **109:** 285–287, 1985.
254. MORROW, A. L., AND CREESE, I.: Characterization of α_1-adrenergic receptor subtypes in rat brain: a reevaluation of ^{3}H-WB 4101 and ^{3}H-prazosin binding. Mol. Pharmacol. **29:** 321–330, 1986.
255. MULLER-SCHWEINITZER, E.: Tissue specific susceptibility of alpha-adrenoceptor mediated vasoconstriction to nifedipine. Naunyn Schmiedebergs Arch. Pharmakol. **324:** 64–69, 1983.
256. NACHSEN, S. A., AND BLAUSTEIN, M. D.: The effects of some organic "calcium antagonists" on calcium influx in presynaptic nerve terminals. Mol. Pharmacol. **16:** 579–586, 1979.
257. NAKAMURA, T., AND UI, M.: Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet-activating protein, pertussis toxin. J. Biol. Chem. **260:** 3584–3593, 1985.
258. NEYLON, C. B., AND SUMMERS, R. J.: ^{3}H-Rauwolscine binding to α_2-adrenoceptors in the mammalian kidney: apparent receptor heterogeneity between species. Br. J. Pharmacol. **85:** 349–359, 1985.
259. NEYLON, C. B., AND SUMMERS, R. J.: Stimulation of α_1-adrenoceptors in rat kidney mediates increasd inositol phospholipid hydrolysis. Br. J. Pharmacol. **91:** 367–376, 1987.
260. NICHOLS, A. J., AND RUFFOLO, R. R., JR.: The relationship between alterations in α_1-adrenoceptor reserve by phenoxybenzamine and benextramine and the sensitivty of cirazoline-induced pressor responses to inhibition by nifedipine. Eur. J. Pharmacol. **126:** 297–301, 1986.
261. NISHIZUKA, Y.: The role of protein kinase C in cell-surface receptor signal transduction and tumor production. Nature (Lond.) **308:** 693–698, 1984.
262. NOCK, B., AND FEDER, H. H.: α_1-Noradrenergic regulation of hypothalamic progestin receptors and guinea pig lordosis behavior. Brain Res. **310:** 77–85, 1984.
263. NOWYCKY, M. C., FOX, A. P., AND TSIEN, R. W.: Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature (Lond.) **316:** 440–443, 1985.
264. NYBORG, N. C. S., AND MULVANY, M. J.: Effect of felodipine, a new dihydropyridine vasodilator, on contractile responses to potassium, noradrenaline, and calcium in mesenteric resistance vessels of the rat. J. Cardiovasc. Pharmacol. **6:** 499–505, 1984.
265. O'BRIEN, J. W., FLAVAHAN, N. A., GRANT, T. L., McGRATH, J. C., AND MARSHALL, R. J.: Influence of blood gases, Ca^{2+} entry blockade, and angiotensin converting enzyme inhibition on pressor responses to α-adrenoceptor agonists: evidence in vivo for subtypes of response independent of receptor subtype? Clin. Sci. **68:** (suppl. 10): 99s–104s, 1985.
266. ORIOWO, M. A.: Effect of calcium entry blockers and divalent cations on noradrenaline-induced contractions of the rat anococcygeus muscle. Arch. Int. Pharmacodyn. Ther. **271:** 45–52, 1984.
267. ORIOWO, M. A., BEVAN, J. A., AND BEVAN, R. D.: Variation in sensitivity of alpha adrenoceptor-mediated contraction of the vascular smooth muscle of rabbit elastic and muscular arteries is related to receptor affinity. J. Pharmacol. Exp. Ther. **241:** 239–244, 1987.
268. PARTINGTON, C. R., EDWARDS, M. W., AND DALY, J. W.: Regulation of cyclic AMP formation in brain tissue by α-adrenergic receptors: requisite intermediacy of prostaglandins of the E series. Proc. Natl. Acad. Sci. USA **77:** 3024–3028, 1980.
269. PEARCE, B., MORROW, C., AND MURPHY, S.: Receptor-mediated inositol phospholipid hydrolysis in astrocytes. Eur. J. Pharmacol. **121:** 231–243, 1986.
270. PEDRINELLI, R., AND TARAZI, R. C.: Calcium entry blockade by nitrendipine and alpha adrenergic responsiveness in vivo: comparison with noncalcium entry blocker vasodilators in absence and presence of phenoxybenzamine pretreatment. J. Pharmacol. Exp. Ther. **233:** 636–642, 1985.
271. PEDRINELLI, R., AND TARAZI, R. C.: Calcium entry blockade by nitrendipine and alpha adrenergic responsiveness in vivo: comparison of systemic vs. local effects. J. Pharmacol. Exp. Ther. **233:** 643–649, 1985.
272. PEIPER, U., GRIEBEL, L., AND WENDE, W.: Activation of vascular smooth muscle of rat aorta by noradrenaline and depolarization: two different mechanisms. Pfluegers Arch. Eur. J. Physiol. **330:** 74–89, 1971.
273. PERNEY, T. M., HIRNING, L. D., LEEMAN, S. E., AND MILLER, R. J.: Multiple calcium channels mediate neurotransmitter release from peripheral neurons. Proc. Natl. Acad. Sci. USA **83:** 6656–6659, 1986.
274. PFEILSCHIFTER, J., AND BAUER, C.: Pertussis toxin abolishes angiotensin II-induced phosphoinositide hydrolysis and prostaglandin synthesis in rat renal mesangial cells. Biochem. J. **236:** 289–294, 1986.
275. PRPIC, V., BLACKMORE, P. F., AND EXTON, J. H.: Phosphatidylinositol breakdown induced by vasopressin and epinephrine in hepatocytes is calcium-dependent. J. Biol. Chem. **257:** 11323–11331, 1982.
276. PURDY, R. E., ASHBROOK, D. W., STUPECKY, G. L., AND WATANABE, M. Y.: Qualitative and quantitative differences between the postsynaptic alpha adrenoceptors of rabbit ear artery and thoracic aorta. J. Pharmacol. Exp. Ther. **224:** 543–551, 1983.
277. PURDY, R. E., AND STUPECKY, G. L.: Characterization of the alpha adrenergic receptor properties of rabbit ear artery and thoracic aorta. J. Pharmacol. Exp. Ther. **229:** 459–468, 1984.

278. PUTNEY, J. W.: Stimulus-permeability coupling: role of calcium in the receptor regulation of membrane permeability. Pharmacol. Rev. **30:** 209–245, 1978.
279. PUTNEY, J. W.: A model for receptor-regulated calcium entry. Cell Calcium **7:** 1–12, 1986.
280. PUTNEY, J. W.: Formation and actions of calcium-mobilizing messenger, inositol 1,4,5-trisphosphate. Am. J. Physiol. **252:** G149–G157, 1987.
281. RAFFA, R. B., TALLARIDA, R. J., AND GERO, A.: Determination of the stimulus-response relationships for three alpha-adrenergic agonists on rabbit aorta. Arch. Int. Pharmacodyn. Ther. **211:** 197–207, 1979.
282. RANDRIANTSOA, A., HEITZ, C., AND STOCLET, J-C.: Functional characterization of postjunctional α-adrenoceptors in rat aorta. Eur. J. Pharmacol. **75:** 57–60, 1981.
283. RANE, S. G., AND DUNLAP, K.: Kinase C activator 1,2-oleoylacetylglycerol attenuates voltage-dependent calcium current in sensory neurones. Proc. Natl. Acad. Sci. USA **83:** 184–188, 1986.
284. RANE, S. G., HOLZ, G. G., AND DUNLAP, K.: Dihydropyridine inhibition of neuronal calcium current and substance P release. Pfluegers Arch. Eur. J. Physiol. **409:** 361–366, 1987.
285. RASMUSSEN, H., AND BARRETT, P. Q.: Calcium messenger system: an integrated view. Physiol. Rev. **64:** 938–984, 1984.
286. REHAVI, M., YAVETZ, B., RAMOT, O., AND SOKOLOVSKY, M.: Regional heterogeneity of two high affinity binding sites for ^{3}H-WB 4101 in mouse brain. Life Sci. **26:** 615–621, 1980.
287. REINHART, P. H., TAYLOR, W. M., AND BYGRAVE, F. L.: The contribution of both extracellular and intracellular calcium to the action of α-adrenergic agonists in perfused rat liver. Biochem. J. **220:** 35–42, 1984.
288. REINHART, P. H., TAYLOR, W. M., AND BYGRAVE, F. L.: The action of α-adrenergic agonists on plasma membrane calcium fluxes in perfused rat liver. Biochem. J. **220:** 43–50, 1984.
289. REYNOLDS, E. E., AND DUBYAK, G. R.: Activation of calcium mobilization and calcium influx by alpha-1 adrenergic receptors in a smooth muscle cell line. Biochem. Biophys. Res. Commun. **130:** 627–632, 1985.
290. RIMELE, T. J., ROOKE, T. W., AARHUS, L. L., AND VANHOUTTE, P. M.: Alpha-1 adrenoceptors and calcium in isolated canine coronary arteries. J. Pharmacol. Exp. Ther. **226:** 668–672, 1983.
291. ROONEY, T. A., AND NAHORSKI, S. R.: Regional characterization of agonist and depolarization induced phosphoinositide hydrolysis in rat brain. J. Pharmacol. Exp. Ther. **239:** 873–880, 1986.
292. RUFFOLO, R. R., JR.: Review: important concepts of receptor theory. J. Auton. Pharmacol. **2:** 277–295, 1982.
293. RUFFOLO, R. R., JR., MORGAN, E. L., AND MESSICK, K.: Possible relationship between receptor reserve and the differential antagonism of alpha-1 and alpha-2 adrenoceptor mediated pressor responses by calcium channel antagonists in the pithed rat. J. Pharmacol. Exp. Ther. **230:** 587–594, 1984.
294. RUFFOLO, R. R., JR., ROSING, E. L., AND WADDELL, J. E.: Receptor interactions of imidazolines. I. Affinity and efficacy for alpha adrenergic receptors in rat aorta. J. Pharmacol. Exp. Ther. **209:** 429–436, 1979.
295. RUFFOLO, R. R., JR., TUROWSKI, B. S., AND PATIL, P. N.: Lack of cross-desensitization between structurally dissimilar alpha-adrenoceptor agonists. J. Pharm. Pharmacol. **29:** 378–380, 1977.
296. RUFFOLO, R. R., JR., AND WADDELL, J. E.: Receptor interactions of imidazolines: α-adrenoceptors of rat and rabbit aortae differentiated by relative potencies, affinities, and efficacies of imidazoline agonists. Br. J. Pharmacol. **77:** 169–176, 1982.
297. RUFFOLO, R. R., JR., WADDELL, J. E., AND YADEN, E. L.: Postsynaptic alpha adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of alpha-2 adrenergic receptors in rat aorta. J. Pharmacol. Exp. Ther. **217:** 235–240, 1981.
298. RUFFOLO, R. R., JR., WADDELL, J. E., AND YADEN, E. L.: Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. J. Pharmacol. Exp. Ther. **221:** 309–314, 1982.
299. RUFFOLO, R. R., JR., YADEN, E. L., AND WADDELL, J. E.: Receptor interactions of imidazolines. V. Clonidine differentiates postsynaptic alpha adrenergic receptor subtypes in tissues from the rat. J. Pharmacol. Exp. Ther. **213:** 557–561, 1980.
300. SAKKIBARA, Y., FUJIWARA, M., AND MURAMATSU, I.: Pharmacological characterization of the alpha-adrenoceptors of the dog basilar artery. Naunyn Schmiedebergs Arch. Pharmakol. **319:** 1–7, 1982.
301. SASTRE, A., GRIENDLING, K. K., RUSHER, M. M., AND MILNOR, W. R.: Relations between alpha adrenergic receptor occupation and contractile response: radioligand and physiological studies in canine aorta. J. Pharmacol. Exp. Ther. **229:** 887–896, 1984.
302. SATTIN, A., RALL, T. W., AND ZANELLA, J.: Regulation of cyclic adenosine 3′5′-monophosphate levels in guinea pig cerebral cortex by interaction of alpha-adrenergic and adenosine receptor activity. J. Pharmacol. Exp. Ther. **192:** 22–29, 1975.
303. SCARBOROUGH, N. L., AND CARRIER, G. O.: Nifedipine and alpha adrenoceptors in rat aorta. I. Role of extracellular calcium in alpha-1 and alpha-2 mediated contraction. J. Pharmacol. Exp. Ther. **231:** 597–602, 1984.
304. SCHAAD, N. C., SCHORDERET, M., AND MAGISTRETTI, P. J.: Prostaglandins and the synergism between VIP and noradrenaline in the cerebral cortex. Nature (Lond.) **328:** 637–640, 1987.
305. SCHMITZ, J. M., GRAHAM, R. M., SAGALOWSKI, A., AND PETTINGER, W. A.: Renal α_1- and α_2-adrenergic receptors: Biochemical and pharmacological correlations. J. Pharmacol. Exp. Ther. **219:** 400–406, 1981.
306. SCHOEPP, D. D., KNIEPPER, S. M., AND RUTLEDGE, C. O.: Norepinephrine stimulation of phosphoinositide hydrolysis in rat cerebral cortex is associated with the α_1-adrenoceptor. J. Neurochem. **43:** 1758–1761, 1984.
307. SCHULTZ, J., AND DALY, J. W.: Adenosine 3′,5′-monophosphate in guinea pig cerebral cortical slices: effects of alpha- and beta-adrenergic agents, histamine, serotonin, and adenosine. J. Neurochem. **21:** 573–580, 1973.
308. SCHUMANN, H. J., GORLITZ, B. D., AND WAGNER, J.: Influence of papaverine, D600, and nifedipine on the effects of noradrenaline and calcium on the isolated aorta and mesenteric artery of the rabbit. Naunyn Schmiedebergs Arch. Pharmakol. **289:** 409–418, 1975.
309. SCHUMANN, H. J., AND LUES, I.: Postjunctional alpha-adrenoceptors in the isolated saphenous vein of the rabbit. Naunyn Schmiedebergs Arch. Pharmakol. **323:** 328–334, 1983.
310. SCHWABE, U., AND DALY, J. W.: The role of calcium ions in accumulations of cyclic adenosine monophosphate elicited by alpha and beta adrenergic agonists in rat brain slices. J. Pharmacol. Exp. Ther. **202:** 134–143, 1977.
311. SCHWARZ, K. R., CARTER, E. A., HOMCY, C. J., AND GRAHAM, R. M.: Agonist interactions at hepatic α_1- and β-adrenergic receptors: affinity state regulation by guanine nucleotides and temperature. Biochemistry **25:** 7782–7788, 1986.
312. SHALABY, I. S., KONGSAMUT, S., FEEDMAN, D., AND MILLER, R. J.: The effect of dihydropyridines on neurotransmitter release from cultured neuronal cells. Life Sci. **35:** 1289–1295, 1985.
313. SHEYS, E. M., AND GREEN, R. D.: A quantitative study of α-adrenergic receptors in the spleen and aorta of the rabbit. J. Pharmacol. Exp. Ther. **180:** 317–325, 1972.
314. SHOGAKIUCHI, Y., KANAIDE, H., KOBAYASHI, S., HISHIMURA, J., AND NAKAMURA, M.: Intracellular free calcium transients induced by norepinephrine in rat aortic smooth muscle cells in primary culture. Biochem. Biophys. Res. Commun. **135:** 9–15, 1986.
315. SIMPSON, P., BISHOPIRC, N., COUGHLIN, S., KARLINER, J., ORDAHL, C., STARKSEN, N., TSAO, T., WHITE, N., AND WILLIAMS, L.: Dual trophic effects of the α_1-adrenergic receptor in cultured neonatal rat heart muscle cells. J. Mol. Cell Cardiol. **18:** 45–58, 1986.
316. SKARBY, T. V. C., ANDERSSON, K. E., AND EDVINSSON, L.: Pharmacological characterization of postjunctional alpha-adrenoceptors in isolated feline cerebral and peripheral arteries. Acta Physiol. Scand. **117:** 63–73, 1983.
317. SKOMEDAL, T., AASS, H., OSNES, J-B., FJELD, N. B., KLINGEN, G., LANGSLET, A., AND SEMB, G.: Demonstration of an alpha adrenoceptor-mediated inotropic effect of norepinephrine in human atria. J. Pharmacol. Exp. Ther. **233:** 441–446, 1985.
318. SLIVKA, S. R., AND INSEL, P. A.: α_1-Adrenergic receptor mediated phosphoinositide hydrolysis and prostaglandin E_2 formation in Madin-Darby canine kidney cells: possible parallel activation of phospholipase C and phospholipase A_2. J. Biol. Chem. **262:** 4200–4207, 1987.
319. SNAVELEY, M. D., AND INSEL, P. A.: Characterization of α-adrenergic receptor subtypes in rat renal cortex: differential regulation of α_1- and α_2-adrenergic receptors by guanyl nucleotides and Na^+. Mol. Pharmacol. **22:** 532–546, 1982.
320. SOMLYO, A. P., AND SOMLYO, A. V.: Vascular smooth muscle. I. Normal structure, pathology, biochemistry, and biophysics. Pharmacol. Rev. **20:** 197–272, 1968.
321. SOMLYO, A. V., AND SOMLYO, A. P.: Electromechanical and pharmacomechanical coupling in vascular smooth muscle. J. Pharmacol. Exp. Ther. **159:** 129–145, 1968.
322. STARKE, K.: α-Adrenoceptor subclassification. Rev. Physiol. Biochem. Pharmacol. **88:** 199–236, 1981.
323. STARKE, K., BOROWSKI, E., AND ENDO, T.: Preferential blockade of presynaptic α receptors by yohimbine. Eur. J. Pharmacol. **34:** 385–388, 1975.
324. STEINBERG, S. F., DRUGGE, E. D., BILEZIKIAN, J. P., AND ROBINSON, R. B.: Acquisition by innervated cardiac myocytes of a pertussis toxin-specific regulatory protein linked to the α_1-receptor. (Wash. DC) **230:** 186–188, 1985.
325. STONE, E. A., AND HERRERA, A. S.: α-Adrenergic modulation of cyclic AMP formation in rat CNS: highest level in olfactory bulb. Brain Res. **384:** 401–403, 1986.
326. STONE, E. A., PLATT, J. E., HERRERA, A. S., AND KIRK, K. L.: Effect of repeated restraint stress, desmethylimipramine, or adrenocorticotropin on the alpha and beta adrenergic components of the cyclic AMP response to norepinephrine in rat brain slices. J. Pharmacol. Exp. Ther. **237:** 702–707, 1986.
327. STORY, D. F., STANDFORD-STARR, C. A., AND RAND, M. J.: Evidence for the involvement of α_1-adrenoceptors in negative feedback regulation of noradrenergic transmitter release in rat atria. Clin. Sci. **68:** (suppl. 10): 111s–115s, 1985.
328. STRADER, C. D., CANDELORE, M. R., RANDS, E., AND DIXON, R. A. F.: β-Adrenergic receptor subtype is an intrinsic property of the receptor gene product. Mol. Pharmacol. **32:** 179–183, 1987.
329. STREB, H., IRVINE, R. F., BERRIDGE, M. J., AND SCHULZ, I.: Release of calcium from a non-mitochondrial intracellular store in pancreatic acinar cells by inosiol-1,4,5-trisphoshate. Nature (Lond.) **306:** 67–69, 1983.
330. STUREK, M., AND HERMSMEYER, K.: Calcium and sodium channels in

spontaneously contracting vascular muscle cells. Science (Wash. DC) **233:** 475–478, 1986.

331. SUGDEN, A. L., SUGDEN, D., AND KLEIN, D. C.: Essential role of calcium influx in the adrenergic regulation of cAMP and cGMP in rat pinealocytes. J. Biol. Chem. **261:** 11608–11612, 1986.
332. SUGDEN, A. L., SUGDEN, D., AND KLEIN, D. C.: α_1-Adrenoceptor activation elevates cytosolic calcium in rat pinealocytes by increasing net influx. J. Biol. Chem. **262:** 741–745, 1987.
333. SUGDEN, D., JR., VANAECEK, J., KLEIN, D. C., THOMAS, T. P., AND ANDERSON, W. B.: Activation of protein kinase C potentiates isoprenaline-induced cyclic AMP accumulation in rat pinealocytes. Nature (Lond.) **314:** 359–361, 1985.
334. TALLARIDA, R. J., SEVY, R. W., HARAKAL, C., BENDRICK, J., AND FAUST, R.: The effect of preload on the dissociation constant of norepinephrine in isolated strips of rabbit thoracic aorta. Arch. Int. Pharmacodyn. Ther. **210:** 67–74, 1974.
335. TERMAN, B. I., SLIVKA, S. R., HUGHES, R. J., AND INSEL, P. A.: α_1-Adrenergic receptor linked guanine nucleotide binding protein in muscle and kidney epithelial cells. Mol. Pharmacol. **31:** 12–20, 1987.
336. TIMMERMANS, P. B. M. W. M., ALI, F. K., KWA, H. Y., SCHOOP, A. M. C., SLOTHORST-GRISDIJK, F. P., AND VAN ZWIETEN, P. A.: Identical antagonist selectivity of central and peripheral alpha-1 adrenoceptors. Mol. Pharmacol. **20:** 295–302, 1981.
337. TIMMERMANS, P. B. M. W. M., BECKERINGH, J. J., VAN ZWIETEN, P. A., AND THOOLEN, M. J. M. C.: Sensitivity of alpha-1 adrenoceptor mediated pressor responses to inhibition by Ca^{2+} entry blockers in the pithed rat: arguments against the role of receptor reserve. J. Pharmacol Exp. Ther. **240:** 864–870, 1987.
338. TIMMERMANS, P. B. M. W. M., DE JONGE, A., VAN MEEL, J. C. A., MATHY, M. J., AND VAN ZWIETEN, P. A.: Influence of nifedipine on functional responses in vivo initiated at α_2-adrenoceptors. J. Cardiovasc. Pharmacol. **5:** 1–11, 1983.
339. TIMMERMANS, P. B. M. W. M., KWA, H. Y., AND VAN ZWIETEN, P. A.: Possible subdivision of postsynaptic alpha-adrenoceptors mediating pressor responses in the pithed rat. Naunyn Schmiedebergs Arch. Pharmakol. **310:** 189–196, 1979.
340. TIMMERMANS, P. B. M. W. M., MATHY, M. J., WILFFERT, B., KALKMAN, H. O., THOOLEN, M. J. M. C., DE JONGE, A., VAN MEEL, J. C. A., AND VAN ZWIETEN, P. A.: Differential effect of calcium entry blockers on alpha-1 adrenoceptor mediated vasoconstriction in vivo. Naunyn Schmiedebergs Arch. Pharmakol. **324:** 239–245, 1983.
341. TIMMERMANS, P. B. M. W. M., AND THOOLEN, M. J. M. C.: Ca^{2+} utilization in signal transformation of alpha-1 adrenergic receptors. *In* The Alpha-1 Adrenergic Receptors, ed. by R. R. Ruffolo, Jr., pp. 113–187, Humana Press, Clifton, NJ, 1987.
342. TIMMERMANS, P. B. M. W. M., THOOLEN, M. J. M. C., MATHY, M. J., WILFFERT, B., DE JONGE, A., AND VAN ZWIETEN, P. A.: Sgd 101/75 is distinguished from other selective alpha-1 adrenoceptor agonists by the inhibition of its pressor responses by calcium entry blockade and vasodilatation in pithed rats and cats. Eur. J. Pharmacol. **96:** 187–192, 1983.
343. TIMMERMANS, P. B. M. W. M., THOOLEN, M. J. M. C., MATHY, M. J., WILFFERT, B., DE JONGE, A., AND VAN ZWIETEN, P. A.: Effects of the irreversible α-adrenoceptor antagonists phenoxybenzamine and benextramine on the effectiveness of nifedipine in inhibiting α_1- and α_2-adrenoceptor mediated vasoconstriction in pithed rats. Naunyn Schmiedebergs Arch. Pharmakol. **329:** 404–413, 1985.
344. TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: The postsynaptic α_2-adrenoceptor. J. Auton. Pharmacol. **1:** 171–183, 1981.
345. TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: α_2-Adrenoceptors: classification, localization, mechanisms, and targets for drugs. J. Med. Chem. **25:** 1389–1401, 1983.
346. TSUJIMOTO, G., BRISTOW, M. R., AND HOFFMAN, B. B.: Identification of alpha-1-adrenergic receptors in rabbit aorta with ^{125}IBE 2254. Life Sci. **34:** 639–646, 1984.
347. TUNG, L.-H., RAND, M. J., DRUMMER, O. H., AND LOUIS, W. J.: Positive chronotropic responses produced by α-adrenoceptors in the pithed rat. J. Auton. Pharmacol. **2:** 217–223, 1982.
348. UHING, R. J., PRPIC, V., JIANG, H., AND EXTON, J. H.: Hormone-stimulated polyphosphoinositide breakdown in rat liver plasma membranes: roles of guanine nucleotides and calcium. J. Biol. Chem. **261:** 2140–2146, 1986.
349. VANACEK, J., SUGDEN, D., WELLER, J., AND KLEIN, D. C.: Atypical synergistic α_1- and β-adrenergic regulation of adenosine 3′,5′-monophosphate and guanosine 3′,5′-monophosphate in rat pinealocytes. Endocrinology **116:** 2167–2173, 1985.
350. VAN BREEMEN, C.: Blockade of membrane calcium fluxes by lanthanum in relation to vascular smooth muscle contractility. Arch. Int. Physiol. Biochim. **77:** 710–718, 1969.
351. VAN BREEMEN, C., AARONSON, P., AND LOUTZENHISER, R.: Sodium-calcium interactions in mammalian smooth muscle. Pharmacol. Rev. **30:** 167–208, 1979.
352. VAN BREEMEN, C., AARONSON, P., LOUTZENHISER, R., AND MEISHERIA, K.: Calcium fluxes in isolated rabbit aorta and guinea pig *Taenia coli* Fed. Proc. **41:** 2891–2897, 1982.
353. VAN BREEMEN, C., HWANG, O., AND CAUVIN, C.: Ca antagonist inhibition of norepinephrine stimulated Ca influx in vascular smooth muscle. *In* Calcium Modulators, ed. by T. Godfraind, A. Albertini, and R. Paoletti, pp. 185–198, Elsevier Biomedical, Amsterdam, 1982.
354. VANHOUTTE, P. M.: Calcium entry blockers, vascular smooth muscle, and hypertension. Am. J. Cardiol. **55:** 17B–23B, 1985.
355. VAN MEEL, J. C. A., DE JONGE, A., KALKMAN, H. O., WILFFERT, B., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Vascular smooth muscle contraction initiated by postsynaptic α_2-adrenoceptor activation is induced by an influx of extracellular calcium. Eur. J. Pharmacol. **69:** 205–208, 1981.
356. VAN MEEL, J. C. A., DE JONGE, A., KALKMAN, H. O., WILFFERT, B., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Organic and inorganic calcium antagonists reduce vasoconstriction in vivo mediated by postsynaptic α_2-adrenoceptors. Naunyn Schmiedebergs Arch. Pharmakol. **316:** 288–293, 1981.
357. VAN MEEL, J. C. A., TOWART, R., KAZDA, S., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Correlation between the inhibitory activities of calcium antagonists on vascular smooth muscle constriction in vitro after K-depolarization and in vivo after α_2-adrenoceptor stimulation. Naunyn Schmiedebergs Arch. Pharmakol. **322:** 34–37, 1983.
358. VAN ZWIETEN, P. A., VAN MEEL, J. C. A., AND TIMMERMANS, P. B. M. W. M.: Pharmacology of calcium entry blockers: interaction with vascular alpha-adrenoceptors. Hypertension **5** (suppl. II): II-8–II-17, 1983.
359. VILA, E., THOOLEN, M. J. M. C., BECKERINGH, J. J., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Lack of effect of D600 on α_1-adrenoceptor mediated contractions of rat isolated anococcygeus muscle. Eur. J. Pharmacol. **106:** 97–105, 1985.
360. VILLALOBOS-MOLINA, R., UC, M., HONG, E., AND GARCIA-SAINZ, J. A.: Correlation between phosphatidylinositol labeling and contraction in rabbit aorta: effect of alpha-1 adrenergic activation. J. Pharmacol. Exp. Ther. **222:** 258–261, 1982.
361. VON TSCHARNER, V., PROD'HOM, B., BAGGIOLINI, M., AND REUTER, H.: Ion channels in human neutrophils activated by a rise in free cytosolic calcium concentration. Nature (Lond.) **324:** 369–372, 1986.
362. WAGNER, J., AND BRODDE, O.-E.: On the presence and distribution of α-adrenoceptors in the heart of various mammalian species. Naunyn Schmiedeberg's Arch. Pharmakol. **302:** 239–254, 1978.
363. WALLACE, M. A., AND FAIN, J. N.: Guanosine 5′-O-thiotriphosphate stimulates phospholipase C activity in plasma membranes of rat hepatocytes. J. Biol. Chem. **260:** 9527–9530, 1985.
364. WALUS, K. M., FONDACARO, J. D., AND JACOBSON, E. D.: Effect of calcium and its antagonists on the canine mesenteric circulation. Circ. Res. **48:** 692–700, 1981.
365. WIKBERG, J. E. S., AKERS, M., CARON, M. G., AND HAGEN, P-O.: Norepinephrine-induced down regulation of alpha-1 adrenergic receptors in cultured rabbit aorta smooth muscle cells. Life Sci. **33:** 1409–1417, 1983.
366. WILFFERT, B., VAN HEININGEN, P. N. M., MATHY, M. J., DE JONGE, A., THOOLEN, M. J. M. C., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Interactions between the putative calcium entry promoter Bay K 8644 and pressor responses produced by alpha-1 and alpha-2 adrenoceptor agonists in the pithed normotensive rat. Naunyn Schmiedebergs Arch. Pharmakol. **328:** 76–82, 1984.
367. WOODS, N. M., CUTHBERTSON, K. S. R., AND COBBOLD, P. H.: Agonist-induced oscillations in cytoplasmic free calcium concentrations in single rat hepatocytes. Cell Calcium **8:** 79–100, 1986.
368. WOODS, N. M., CUTHBERTSON, K. S. R., AND COBBOLD, P. H.: Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. Nature (Lond.) **319:** 600–602, 1987.
369. YATANI, A., CODINA, J., IMOTO, Y., REEVES, J. P., BIRNBAUMER, L., AND BROWN, A. M.: Direct regulation of mammalian cardiac calcium channels by a G protein. Science (Wash. DC) **238:** 1288–1292, 1987.
370. ZATZ, M.: Denervation supersensitivity of the rat pineal to norepinephrine-stimulated ^{3}H-inositide turnover revealed by lithium and a convenient procedure. J. Neurochem. **45:** 95–100, 1985.
371. ZATZ, M.: Phorbol esters mimic α-adrenergic potentiation of serotonin-N-acetyltransferase induction in the rat pineal. J. Neurochem. **45:** 637–639, 1985.