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S.-O. Chua is on leave from the University Sains Malaysia, Penang, Malaysia.

Heterogeneity of Biochemical Actions among Vasodilators

FORREST C. GREENSLADE, CYNTHIA K. SCOTT ˣ, KATHRYN L. NEWQUIST, KATHRYN M. KRIDER, and MARK CHASIN

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Abstract □ Thirty-four vasodilators were screened in several *in vitro* biochemical assays related to smooth muscle excitation–contraction coupling, binding to β_1^-, β_2^-, and α-adrenergic receptors, inhibition of phosphodiesterase activity, and antagonism of calcium accumulation. Isoproterenol and perhexiline only exhibited binding to β-adrenergic sites. Ergocryptine, tolazoline, and amotriphene only bound to α-adrenergic receptors. Leniquinsin, papaverine, proquazone, dioxyline, hoquizil, quazodine, and theophylline were active only as phosphodiesterase inhibitors. Isoxsuprine, nylidrin, and bencyclane bound to α- and β-receptors. Pentoxifylline bound to β_1-sites and inhibited phosphodiesterase. Cyclandelate bound to β_2-sites and blocked calcium accumulation. Cinnarizine and flunarizine antagonized calcium accumulation and bound to α-sites. Prazosin bound to α-sites and inhibited phosphodiesterase. Ethaverine and dipyridamole were inhibitors of phosphodiesterase and calcium accumulation. Nafronyl bound to β_2- and α-sites and antagonized calcium accumulation. Mebeverine bound to β_2- and α-receptors and inhibited phosphodiesterase activity and calcium accumulation. Verapamil bound to α-sites, and blocked phosphodiesterase and calcium accumulation. Quinazosin bound to β_2- and α-receptors and antagonized both phosphodiesterase activity and calcium accumulation. Vasodilators that were inactive in all assays included niacin, nicotinyl alcohol, inositol nicotinate, amyl nitrite, sodium nitroprusside, diazoxide, hydralazine, and protoveratrine. Vasodilators should not be considered as a single drug class since they act on various mechanisms related to coupling of neuronal excitation to muscular contractility.

Keyphrases □ Vasodilators—biochemical mechanisms □ Adrenergic receptors—α, β_1, and β_2, effect of various vasodilators □ Calcium—effect of various vasodilators on intracellular accumulation, red blood cells □ Phosphodiesterase—inhibition by various vasodilators

The treatment of cardiovascular diseases such as hypertension (1, 2), angina pectoris, cardiac failure (3, 4), and certain peripheral vascular diseases (1, 5, 6) utilizes drugs that produce vasodilation. Vasodilation results from an increase in the internal diameter of blood vessels, presumably through relaxation of vascular smooth muscle. Significant progress has been made in elucidating the biochemical mechanisms controlling smooth muscle contractility. Molecular events in that process include the interaction of catecholamines with specific adrenergic receptors of the smooth muscle cell membrane, the activities of enzymes that regulate cellular levels of cyclic AMP, the control of cellular calcium-ion transport and storage, and the interaction of calcium with the contractile proteins of the smooth muscle cell (7–9).

By using this concept of excitation–contraction coupling in smooth muscle as a working hypothesis, the mechanism of action of various vasodilators has been studied. Thirty-four vasodilators (1, 2, 5, 10, 11) were tested in three types of *in vitro* screening models: (*a*) binding to α- and β-adrenergetic receptor sites, (*b*) inhibition of cyclic AMP phosphodiesterase, and (*c*) inhibition of intracellular calcium accumulation. The results suggest that vasodilator drugs interact with a variety of distinct events in the excitation–contraction coupling mechanisms of vascular smooth muscle.

EXPERIMENTAL

Adrenergic Radioreceptor Assays—*β-Adrenergic Receptor Binding Assays*—Sarcolemma-enriched membranes from guinea pig hearts were prepared as described previously (12). Guinea pigs were killed by cervical dislocation, and the hearts were immediately placed in ice-cold physiological saline. Hearts (20–25 g) were minced in 10 volumes of 2.5 m*M* imidazole buffer (pH 7.4) containing 0.1 m*M* ethylenediaminetetraacetic acid (I) and briefly homogenized with a tissue homogenizer. The homogenate was centrifuged for 1 min at 22×*g*.

The supernatant fraction was rehomogenized and recentrifuged five times. The pooled supernatant fractions were passed through three layers of cheesecloth and centrifuged at 1600×*g* for 20 min. The resulting pellet was resuspended in 200 ml of 10 m*M* [tris(hydroxymethyl)aminomethane hydrochloride] buffer (II), pH 7.5, containing 0.1 *M* sucrose and homogenized using a conical polytef[1]/glass tissue grinder. The homogenate was centrifuged at 1600×*g* for 20 min.

These resuspension, homogenization, and centrifugation steps were then repeated. The pellet was resuspended in 50 ml of 0.4 *M* sucrose in 10 m*M* II (pH 7.5) and osmotically shocked by slow dilution into 1 liter of 0.25 m*M* I in 5 m*M* II (pH 7.0) buffer. The membranes were collected by centrifugation at 1600×*g* for 30 min, and the pellet was suspended in 20 ml of 0.4 *M* sucrose in 10 m*M* II (pH 7.5). The suspension was diluted with three volumes of a 13 m*M* II buffer (pH 7.0) containing 1.3 *M* KCl, 2.6 m*M* I, and 0.4 *M* sucrose. After stirring for ~16 hr at 4°, the extract was diluted with an equal volume of buffer [1 *M* KCl–2 m*M* I–10 m*M* II (pH 7.0)]. After centrifugation at 1600×*g* for 30 min, the pellet was washed three times in 100 ml of 0.1 *M* sucrose in 10 m*M* II (pH 7.0) buffer. The final pellet was suspended in 0.1 *M* sucrose and stored in liquid nitrogen.

Bronchial tree membranes were prepared from either fresh guinea pig lungs or lungs stored at −100°. Bronchial trees were exposed by combing through the lungs several times with a steel animal hairbrush to strip them of alveolar tissue. Extraneous tissue was removed by scraping with a scalpel. The bronchial trees were placed in a small volume of ice-cold II (pH 7.4) containing 1 m*M* $MgCl_2$ and 0.25 *M* sucrose. Then they were finely minced and homogenized by four 10-sec bursts with a tissue homogenizer.

The homogenate was filtered through a single layer of cheesecloth, and the filtrate was centrifuged for 10 min at 400×*g*. The pellet was discarded, and the supernate was centrifuged for 10 min at 20,000×*g*. The resulting pellet was washed in 50 m*M* II (pH 7.5) containing 10 m*M* $MgCl_2$ and centrifuged at 28,000×*g* for 10 min. The pellet was resuspended in 75 m*M*

[1] Teflon.

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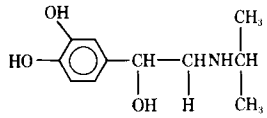
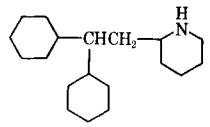
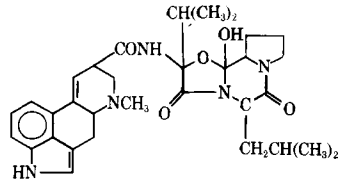
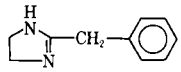
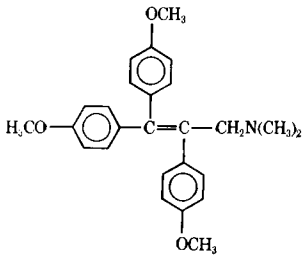
Table I—Vasodilators with Affinity for β-Adrenergic Binding Sites

Compound	Structure	IC_{50}, μM: Adrenergic Receptor Binding β_{1}(Heart)[a]	β_2(Lung)[b]	(Uterus)[c]	PDE[d]	Ca^{2+} [e]
Propranolol	Control	0.05	0.07	100	—[f]	—
Atenolol	Control	5.5	30	—	—	—
Isoproterenol		0.6	1.5	—	—	—
Perhexiline		10	4	—	—	—

[a] Competition for binding of [^{3}H]dihydroalprenolol to specific binding sites on guinea pig heart membranes. [b] Competition for binding of [^{3}H]dihydroalprenolol to specific binding sites on guinea pig lung membranes. [c] Competition for binding of [^{3}H]dihydroergocryptine to specific binding sites on rabbit uterine membranes. [d] Inhibition of rat brain phosphodiesterase activity. [e] Inhibition of $^{45}Ca^{2+}$ accumulation in human red blood cells. [f] Indicates no activity at highest concentration tested (150 μM).

Table II—Vasodilators with Affinity for α-Adrenergic Binding Sites [a]

Compound	Structure	IC_{50}, μM: Adrenergic Receptor Binding β_1 (Heart)	β_2 (Lung)	α (Uterus)	PDE	Ca^{2+}
Phentolamine	Control	—	—	0.025	—	—
Phenoxybenzamine	Control	—	—	0.15	—	3
Ergocryptine		—	—	0.06	—	—
Tolazoline		—	—	4	—	—
Amotriphene		—	—	150	—	—

[a] See footnotes to Table I.

II (pH 8.1) containing 25 m*M* $MgCl_2$ and collected by centrifugation at 28,000×*g*. The final pellet was suspended in the same buffer and stored in liquid nitrogen.

The β-adrenergic binding assays were conducted according to the method of Mukherjee *et al.* (13). The frozen membrane preparation was thawed and maintained on ice. The incubation mixture contained 25 μl of the test drug, 50 μl of [^{3}H]dihydroalprenolol[1] (40,000 cpm; 9 nmoles), and 100 μl of the membrane preparation. A 15-min incubation at 37° was initiated by the addition of the membrane preparation and vortexing. The incubation was terminated by adding 5 ml of ice-cold 75 m*M* I containing 25 m*M* $MgCl_2$ (pH 8.1) and immediately collecting the membranes on glass fiber filter disks. The membranes were washed five times with 5 ml of the same ice-cold buffer. The filter disks were dried in an oven and counted in a scintillation solution[2]. Along with each set of test compounds, incubations were conducted with 1.5×10^{-4} *M* propranolol. Binding of [^{3}H]dihydroalprenolol at this high concentration of propranolol was considered to represent nonspecific binding to β-adrenergic receptor sites.

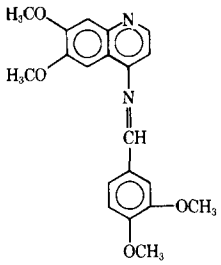
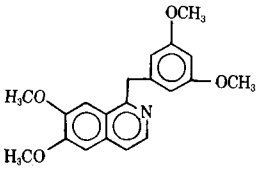
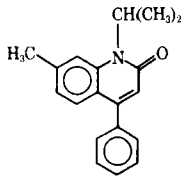
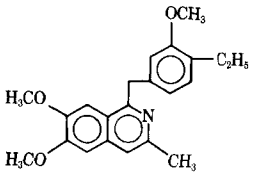
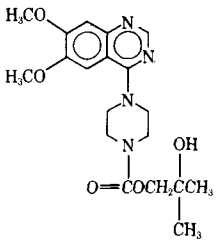
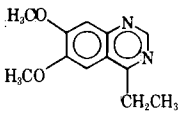
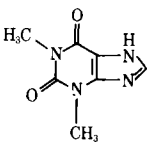
α-Adrenergic Receptor Binding Assay—Membranes were prepared from frozen type II mature rabbit uteri according to the method of Willliams *et al.* (14). After thawing, fat was removed and endometrial cells were scraped free with a scalpel. Uteri were sliced, finely minced with scissors in a solution containing 0.25 *M* sucrose, 5 m*M* II (pH 7.4), and 1 m*M* $MgCl_2$, and then homogenized four times. After filtration through cheesecloth, the homogenate was centrifuged at 400×*g* for 10 min. The supernatant liquid was then centrifuged for 10 min at 39,000×*g*. The resulting pellet was homogenized using a polytef/glass tissue grinder in 50 m*M* II (pH 7.4) containing 10 m*M* $MgCl_2$. The resuspended pellet was centrifuged for 10 min at 39,000×*g*, and the resulting pellet was washed with the same buffer. The final pellet was homogenized in the same buffer and used fresh in the binding assay.

The incubation consisted of 25 μl of test drug, 50 μl of [^{3}H]dihydroergocryptine[3] (~40,000 cpm, 2 pmoles), and 100 μl of the membrane preparation. A 15-min incubation, conducted at 25°, was terminated by addition of 5 ml of room temperature 10 m*M* $MgCl_2$ in 50 m*M* II (pH 7.5). Membranes were collected by fiber filtration and washed with 5 ml of the same buffer at room temperature. Phentolamine at 1.5×10^{-4} *M* was used to define nonspecific binding to the membranes.

[2] Aquasol, New England Nuclear.

[3] 9,10-[9,10-^{3}H(N)], specific activity 24 Ci/nmole, New England Nuclear.

Table III—Vasodilators that Inhibit Phophodiesterase Activity [a]

Compound	Structure	IC_{50}, μM					
		Adrenergic Receptor Binding			PDE		
		β_1 (Heart)	β_2 (Lung)	α (Uterus)	(Brain)	(Artery)	Ca^{2+}
Etazolate	Control	—	—	—	4	NT[b]	—
Leniquinsin		—	—	—	2.8	7.6	—
Papaverine		—	—	—	3.1	NT[b]	—
Proquazone		—	—	—	4.4	19	—
Dioxyline		—	—	—	5.4	1.1	—
Hoquizil		—	—	—	18	15	—
Quazodine		—	—	—	20	48	—
Theophylline		—	—	—	240	NT[b]	—

[a] See footnotes to Table I. [b] Not tested.

Phosphodiesterase Assay—Phosphodiesterase was prepared from rat brain or Cynomolgus monkey abdominal artery by the method of Chasin and Harris (15). Tissues were homogenized in 5–10 volumes of 0.05 *M* II (pH 7.5), and the homogenates were centrifuged for 20 min at 39,000×*g*. The supernate was adjusted to 50% saturation by slow addition of an equal volume of neutral, saturated ammonium sulfate. The precipitate was allowed to form for 1 hr in an ice bath and was collected by centrifugation at 39,000×*g* for 20 min. The precipitate was dissolved in a minimum volume of 0.05 *M* II (pH 7.5) and dialyzed overnight against 20 volumes of the same buffer. The enzyme preparation was stored at 4°.

The enzyme inhibition assay was conducted in plastic scintillation vials. A small drop was produced in the bottom of the vial, which contained 50 μl of II, 50 μl of the [^{3}H]cyclic AMP substrate solution, 50 μl of test drug, and 50 μl of diluted enzyme preparation in 1 mg of bovine serum albumin and 1 mg of snake venom nucleotidase/ml of 0.05*M* II buffer at pH 8.0. The vials were incubated for 10 min at 37° with vigorous shaking. The reaction was terminated by the addition of 0.5 ml of washed anion-exchange resin (200–400 mesh, 50% settled volume).

The phosphodiesterase activity was measured as [^{3}H]adenosine using a modified Bray's scintillation cocktail.

Calcium Transport Assay—The assay for inhibition of calcium accumulation was conducted as described by Scott *et al.* (16). Blood was obtained in heparin from healthy human volunteers, and the red blood

cells were collected by centrifugation at 270×*g*. The cells were washed four times and resuspended in 10 m*M* II (pH 7.3) containing 135 m*M* NaCl, 4.5 m*M* KCl, and 15 m*M* chlorine chloride (III). The cells (400 μl packed red blood cells), 10 μl of test drug solution, and 490 μl of III were preincubated in 12 × 75-mm plastic tubes at 24° for 1 hr. A 100-μl solution of [^{45}Ca]calcium chloride[4] (~1 × 10^6 cpm, specific activity 9.85 mCi/mg) was added, and the tubes were incubated with moderate shaking for 30 min at 37°.

The incubation was terminated by addition of 2 ml of 0.1 m*M* lanthanum chloride in III. Tubes were centrifuged at 270×*g* for 10 min, and the supernate was removed. The cells were washed in 2 ml of buffer and recentrifuged, and the supernate was again removed. Each tube was vortexed, and 200 μl was transferred to a glass scintillation vial. A 200-μl aliquot of 60% perchloric acid was added, the vials were shaken, and then 400 μl of 50% hydrogen peroxide was added with shaking. The vials were heated to 70–80° for 30 min. After cooling, 8 ml of ethylene glycol monomethyl ether was added to each vial, followed by 10 ml of a toluene-phosphor solution (6g of 2,5-diphenyloxazole/liter of scintillation grade toluene). Radioactivity was measured by liquid scintillation spectroscopy.

Drugs—Drugs used as standards in these assays included niacin[5], pentoxifylline[6], dipyridamole[7], amiquinsin[8], leniquinsin[8], dioxyline[9], protoveratrine[9], amylnitrite[9], nicotinyl alcohol[10], sodium nitroprusside[10], cyclandelate[11], cinnarizine[12], flunarizine[12], verapamil[13], nafronyl[14], quazodine[15], ethaverine[16], prazosin[17], quinazosin[17], hoquizil[17], mebeverine[18], diazoxide[19], etazolate hydrochloride[20], phenoxybenzamine[21], bencyclane[22], papaverine[23], nylidrin[23], and amotriphene[24]. Other drugs were obtained commercially.

Drugs were dissolved immediately prior to assay in distilled water, using dimethyl sulfoxide or dimethylformamide as solubilization aids if necessary.

RESULTS

Table I shows the compounds that exhibited binding only to β-adrenergic receptors. Two control compounds were run as assay validation standards. In the competition assays using guinea pig heart and lung membranes and [^{3}H]dihydroalprenolol as the radioligand, the nonselective β-adrenergic antagonist propranolol had high binding affinity. The cardioselective β-blocker atenolol had lower affinity for sites on both heart and lung membranes than did propranolol, the affinity being higher for heart membrane binding sites than for lung membrane sites. The binding sites on guinea pig heart and lung membranes labeled by [^{3}H]-dihydroalprenolol have been characterized as β_1- and β_2-adrenergic, respectively (17).

Two vasodilators exhibited binding affinity for β-adrenergic receptors. Isoproterenol had the highest affinity for both β_1- and β_2-receptors. Perhexiline had lower affinity. Neither compound showed measurable affinity for α-adrenergic receptors. The compounds did not inhibit cyclic AMP phosphodiesterase activity, nor did they block Ca^{2+} accumulation.

Table II shows the vasodilator compounds that bound only to α-adrenergic binding sites. The standard α-antagonist phentolamine had high affinity, and phenoxybenzamine also bound to α-sites. In addition, phenoxybenzamine inhibited the accumulation of calcium 45 into erythrocytes. Ergocryptine and tolazoline also had relatively high affinity for α-adrenergic receptors, while amotriphene exhibited marginal affinity. The three compounds were inactive in the other assays.

Table III lists the active compounds in the phosphodiesterase inhibition assay. The potent phosphodiesterase inhibitor etazolate hydrochloride served as a control standard. Leniquinsin was the most potent of the vasodilators tested with rat brain phosphodiesterase. Papaverine, proquazone, and dioxyline were slightly less potent. Hoquizil and quazodine were less potent, and theophylline was the least potent of the active agents tested. All active compounds tested inhibited phosphodiesterase activity in Cynomolgus monkey abdominal artery. This group of vasodilators was inactive in all other assays.

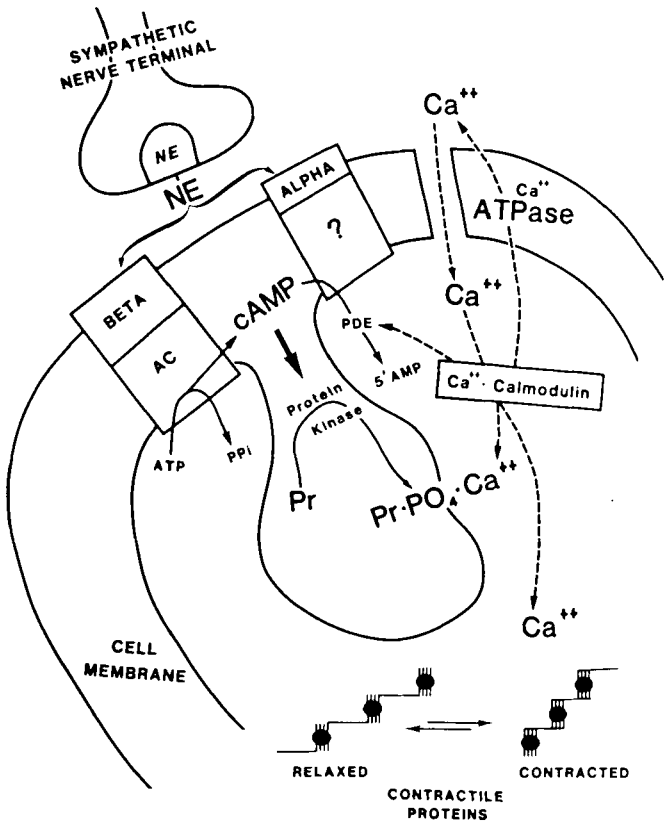


Figure 1—*Possible mechanisms of vascular smooth muscle excitation–contraction coupling.*

Thirteen vasodilators tested were active in more than one assay (Table IV). Isoxyprine, nylidrin, and bencyclane bound to β_1-, β_2-, and α-adrenergic receptors. Pentoxifylline had a low level of affinity for β_1-receptors, and it also inhibited phosphodiesterase activity. Cyclandelate bound to β_2-adrenergic receptors and inhibited accumulation of calcium.

Two compounds, cinnarizine and flunarizine, inhibited accumulation of calcium 45 ion in human red blood cells. Both compounds exhibited some affinity for α-sites. As previously reported (18, 19), prazosin bound to α-receptors. Ethaverine and dipyridamole were phosphodiesterase inhibitors and also inhibited calcium 45 ion accumulation.

Nafronyl was active in three assays. It had low binding affinity for β_2- and α-receptors and inhibited calcium accumulation. Mebeverine also bound to β_2- and α-receptors and inhibited phosphodiesterase activity. Verapamil bound to α-receptors and inhibited both phosphodiesterase activity and calcium 45 ion accumulation.

One compound, quinazosin, was active in four of the five assays. Quinazosin bound to β_2- and α-receptors. It also inhibited phosphodiesterase activity and calcium 45 ion accumulation.

Niacin, nicotinyl alcohol, inositol niacinate, amyl nitrite, sodium nitroprusside, diazoxide, hydralazine, and protoveratrine were inactive in all five assays at the highest concentration listed (150 μ*M*).

DISCUSSION

Vasodilation results from relaxation of vascular smooth muscle and leads to enhanced blood flow and/or reduced peripheral resistance (4, 20). Pharmacologically, vasodilators have been classed in two groups. One group acts indirectly on sympathetic innervation to the arterioles, thereby relieving functional vasoconstriction, and the other acts directly on the smooth muscle of the arterioles. Drugs classically categorized as sympathetic depressants include centrally acting agents such as the dihy-

[4] New England Nuclear.
[5] Supplied by Abbott Laboratories.
[6] Suppliedby Albert-Roussel.
[7] Supplied by Boehringer-Ingelheim.
[8] Supplied by Eaton.
[9] Supplied by Eli Lilly.
[10] Supplied by Hoffmann–La Roche.
[11] Supplied by Ives.
[12] Supplied by Janssen.
[13] Sujpplied by Knoll.
[14] Supplied by Lipha.
[15] Supplied by Mead-Johnson.
[16] Supplied by Parke-Davis.
[17] Supplied by Charles Pfizer.
[18] Supplied by Philips Duphar.
[19] Supplied by Schering-Plough.
[20] Supplied by Squibb.
[21] Supplied by Smith Kline and French.
[22] Supplied by Thiemann.
[23] Supplied by U.S. Vitamin.
[24] Supplied by Winthrop.

Table IV—Vasodilators with Multiple Activities [a]

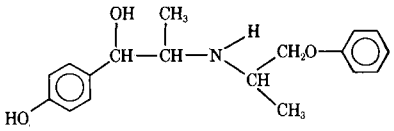
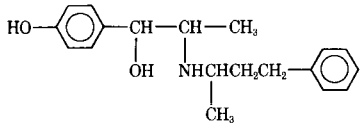
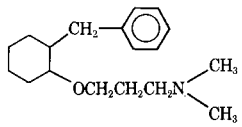
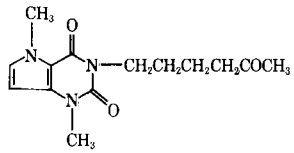
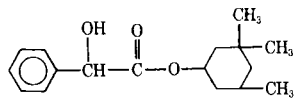
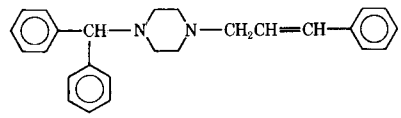
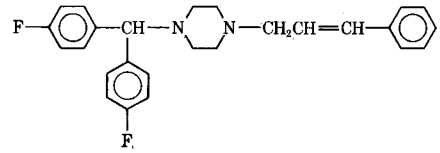
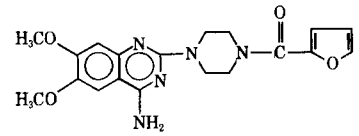
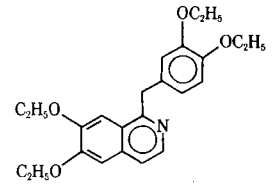
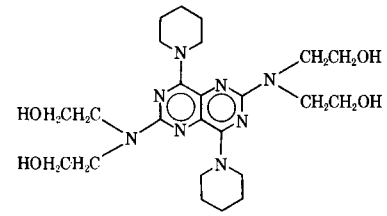
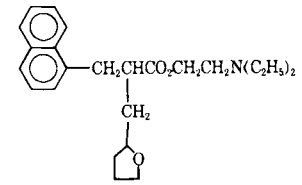
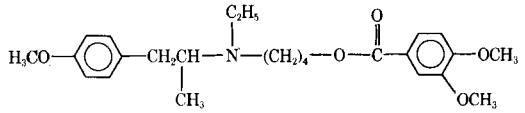
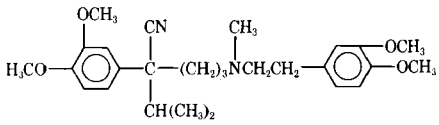
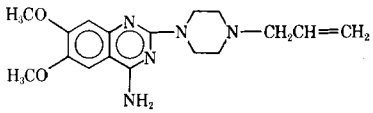
Compound	Structure	IC_{50}, μM					
		Adrenergic Receptor Binding			PDE		
		β_1 (Heart)	β_2 (Lung)	α (Uterus)	(Brain)	(Artery)	Ca^{2+}
Isoxsuprine		15	57	3	—	NT[b]	—
Nylidrin		20	10	50	—	NT[b]	—
Bencyclane		3.5	1.5	15	—	NT[b]	—
Pentoxifylline		85	—	—	48	NT[b]	—
Cyclandelate		—	16	—	—	NT[b]	31
Cinnarizine		—	—	7	—	NT[b]	19
Flunarizine		—	—	25	—	NT[b]	46
Prazosin		—	—	1.0	26	30	—
Ethaverine		—	—	—	1.5	1.6	11
Dipyridamole		—	—	—	4.2	20	5.6
Mafronyl		—	75	50	—	NT[b]	2.5

Table IV—*Continued*

Compound	Structure	IC$_{50}$, μM Adrenergic Receptor Binding β_1 (Heart)	β_2 (Lung)	α (Uterus)	PDE (Brain)	(Artery)	Ca^{2+}
Nebeverine		—	10	20	74	100	—
Verapamil		—	—	2.5	28	64	87
Quinazosin		—	15	1.5	84	140	13

[a] See footnotes to Table I. [b] Not tested.

droergot alkaloids, ganglionic blocking drugs (e.g., pentolinium and mecamylamine), and agents such as guanethidine, which block norepinephrine release at neuroeffector junctions. Drugs categorized as acting on smooth muscle include β-adrenergic stimulating agents such as isoproterenol, isoxsuprine, and nylidrin and α-adrenergic blocking agents such as phenoxybenzamine and tolazoline. Other direct acting vasodilators presumably act on contractile mechanisms of the smooth muscle cell (5).

The biochemical mechanisms that underlie the coupling of sympathetic nervous excitation and smooth muscle contractility are beginning to be understood. Figure 1 shows an hypothesized pathway involving a complex series of biochemical events (7, 8, 21–23), by which adrenergic neurotransmitters regulate smooth muscle contractility. With respect to vasodilation, the following pathway is proposed:

1. Norepinephrine binds to specific β-adrenergic sites on the muscle cell surface.
2. The increased activity of a closely coupled enzyme, adenylate cyclase, elevates cellular cyclic AMP levels.
3. The cellular content of cyclic AMP regulates the activity of a second enzyme, cyclic AMP-dependent protein kinase, which functions to phosphorylate (and thus activates) certain proteins in the sarcoplasmic reticulum. The activated proteins in the sarcoplasmic reticulum control the storage and release of Ca^{2+} ions, which ultimately regulate the contraction of the actin–myosin fibrils of the muscle cell.
4. The heat-stable protein calmodulin binds calcium and activates phosphodiesterase to return cellular cyclic AMP levels back to normal. Simultaneously, calmodulin activates the calcium-stimulated ATP phosphohydrolase (the so-called calcium pump) to return intracellular levels of calcium to baseline levels.

The biochemical events elicited by stimulating α-adrenergic sites are less well understood. Three hypotheses have been proposed:

1. α-Receptor stimulation may inhibit adenylate cyclase or stimulate cyclic AMP phosphodiesterase activity, leading to a decrease in cellular cyclic AMP levels.
2. α-Receptor stimulation causes increased guanylate cyclase activities and increased cyclic GMP levels, leading to physiological actions opposing cyclic AMP.
3. α-Receptors function as calcium ionophores, resulting in increased Ca^{2+} influx.

Most of the vasodilators tested were active in one or more of the assays and thus might be expected to act at some specific point in the described excitation-contraction coupling pathways for vascular smooth muscle. The compounds that were inactive in these assays may act on related mechanisms. For example, papaverine, nitroglycerin, diazoxide, and hydralazine all increased the cyclic AMP content in vascular smooth muscle (24). Furthermore, Chidsey and Gottlieb (20) reviewed studies that suggested that certain vasodilators (diazoxide, minoxidil, and sodium nitroprusside) interfere with some calcium movements responsible for initiation or maintenance of contractile state. These vasodilators might alter the availability of calcium ion to contractile proteins through compartmentalization rather than modification of cellular content.

Several vasodilators exhibit multiple activities. Nylidrin and isoxsuprine, vasodilators classically described as β-adrenergic stimulants, showed affinity for β-receptors. However, these compounds also exhibited affinities for α-receptors. The multiple activities of these drugs is consistent with earlier work (25), which showed that isoxsuprine relaxed a variety of nonvascular smooth muscles through mechanisms independent of β-receptors. These investigators suggested that, in addition to its sympathomimetic action, isoxsuprine may have a papaverine-like, direct effect as well as an adrenergic blocking action. Although radioreceptor assays do not distinguish between agonists and antagonists, these results suggest that this compound more likely acts by blocking α-adrenergic receptors than by inhibiting phosphodiesterase activity. Furthermore, work reviewed by Coffman (6) indicated that the effects of isoxsuprine and nylidrin are not completely antagonized by β-adrenergic blocking agents.

Previous findings (18) indicate that prazosin interacts with both the active site of phosphodiesterase and with α-receptors (18). Subsequent data suggest that prazosin binds to two different populations of binding sites, possibly relating to pre- and postsynaptic α-receptors (19).

The α-blocking agent phenoxybenzamine also was found to be an inhibitor of Ca^{2+} accumulation. The acknowledged calcium antagonist verapamil (26) also exhibited affinity for α-receptors, as well as phosphodiesterase inhibition. The finding that several vasodilators exhibited activity in two of three assays (α-receptor, phosphodiesterase inhibition, and calcium accumulation) suggests a close relationship of three biochemical steps. On the other hand, the finding that some vasodilators were only active in one of these three assays supports the contention that these are closely related but independent mechanisms.

The mechanisms closest to the control of contractile protein function evidently involve the transport, storage, and cellular content of calcium ion. Cinnarizine and flunarizine, for example, inhibit accumulation of calcium ion in human red blood cells (18) and have been reported to produce long-lasting inhibition of calcium-induced contraction in isolated vascular preparations of rat and rabbit (11). Other vasodilators such as papaverine, nafronyl, bencyclane, cyclandelate, dihydroergotamine, xanthinol niacinate, and pentoxifylline were reported by the same investigators to be less potent as inhibitors of Ca^{2+}-induced vasospasms. In this regard, flunarizine appears to possess a degree of selectivity in that (in contrast to the majority of reports) it had little effect on spontaneous myogenic activity (11). Flunarizine also prevents blood hyperviscosity induced by ischemic occlusion (26, 27), an effect apparently related to changes in red blood cell flexibility (28). This drug's vasodilator activity and its effects on red blood cell deformability both probably result from inhibition of Ca^{2+} accumulation (16).

With respect to vasodilation, drug actions in the smooth muscle cell resulting in reduced Ca^{2+} available for interaction with the troponin–actin–myosin complex lead to relaxation of the muscle and dilation of the blood vessel.

The biochemical screening models used in this study were predicted on our working hypothesis and were established to ascertain whether vasodilator drugs might have their effects on specific points in these pathways. Several vasodilators previously were shown to act through cyclic AMP-related mechanisms. Papaverine, for example, is a well-

accepted model compound of a vasodilator acting through cyclic AMP phosphodiesterase inhibition (29, 30), and isoproterenol has been shown to elevate cyclic AMP levels through stimulation of adenylate cyclase. It is clear that elevated intracellular cyclic AMP levels can lead to vasodilation and that compounds that interact with either the α-adrenergic receptor or cyclic AMP phosphodiesterase may be potential vasodilators.

The presence of calcium is a necessary component of smooth muscle contraction. Several workers showed that agents that normally contract vascular smooth muscle *in vitro* cannot cause contraction when calcium is omitted from the incubation or when calcium accumulation into the tissue is blocked. Therefore, agents capable of inhibiting calcium accumulation (*e.g.* flunarizine) may act by preventing vasoconstriction and result in a net vasodilation *in vivo* (31).

It is not certain how directly the results using biochemical screening models can be extrapolated to *in vivo* conditions involving vascular smooth muscle. However, several general findings provide a basis for further experimentation and future working hypotheses.

Vasodilators must be considered a heterogeneous drug class. For example, from a therapeutic point of view, vasodilators used in the treatment of hypertension and peripheral vascular disease require different pharmacological properties. In the treatment of peripheral vascular disease, reduction of blood pressure is undesirable, whereas it is obviously a criterion for vasodilators used in hypertension (1). The optimal requirements for peripheral and cerebral vasodilators may also differ.

The actions of vasodilators clearly involve heterogeneous physiological and biochemical mechanisms related to smooth muscle contractility. It is proposed that both the therapeutic utility and side-effect pattern of a vasodilator will depend on the specific profile of the excitation–contraction coupling mechanisms altered by the drug. Additional research on this series of drugs and on future compounds will provide more specific criteria for classifying vasodilator drugs.

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Liposome Disposition *In Vivo* II: Dose Dependency

MARK E. BOSWORTH and C. ANTHONY HUNT ˣ

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Abstract □ The dose-dependent disposition of extruded multilamellar (diameter ~1 μm) negatively charged liposomes containing entrapped [^{14}C]inulin was studied in mice. Mice received 1500, 300, or 15 μmoles of liposomal lipid/kg iv. Carbon 14 levels were measured in the blood, liver, spleen, and carcass for 72 hr. A pronounced saturation effect, consistent with the known dose behavior of other colloids, was seen at early times; it was manifested by higher dose values in the blood and spleen but by lower liver values as the dose increased. This dose effect was attenuated in the liver but was maintained in the spleen at later times, and percent dose values approached plateau values in all tissues for all doses at later times. [^{14}C]Inulin was used as the liposomal marker because of its inability to enter cells (or, presumably, leave them if delivered there by liposomes) in its free form. An early decline in carbon 14 levels (over the first 48 hr) was seen in the liver for the low and medium doses. Because of the known ability of blood factors to cause liposomes to leak their contents, this decline was interpreted as being a loss of [^{14}C]inulin from extracellularly bound liposomes during this period. Moreover, the plateau carbon 14 levels at later times were interpreted as approximating the true level of intracellular inulin delivery by the liposomes.

Keyphrases □ Liposomes—disposition *in vivo*, dose dependency □ Disposition—of liposomes, dose dependency □ Pharmacokinetics—liposome disposition *in vivo*, dose dependency

Recently, there has been considerable interest in liposomes (phospholipid vesicles) as an *in vivo* drug delivery system. There are now ample data showing that liposome encapsulation can drastically alter the blood kinetics and tissue distribution of numerous compounds. Their potential uses in drug therapy, which were recently reviewed

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