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STUDIES ON THE MECHANISM OF ACTION OF VARIOUS VASODILATORS

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1 The vascular relaxant effects of histamine, adenosine, isoprenaline nitroglycerine, papaverine and 3-isobutyl-l-methylxanthine (IBMX) were assessed individually, in strips of rabbit renal artery moderately contracted with noradrenaline (NA) in the absence or presence of phosphodiesterase inhibitors (papaverine and IBMX) or verapamil, a Ca^{2+} antagonist.

2 The vasodilator effect of histamine was potentiated by papaverine (6.1×10^{-7} M) and IBMX (4.4×10^{-5} M) but inhibited dose-dependently by verapamil (5.1 and 51.0×10^{-7} M).

3 Adenosine-induced vascular relaxations were greatly increased in the presence of papaverine (6.1×10^{-7} M) but significantly reduced in the presence of IBMX (4.4×10^{-5} M) or verapamil (5.1 and 51.0×10^{-7} M).

4 The vasodilatation produced by isoprenaline was increased in the presence of IBMX (4.4×10^{-5} M) or papaverine (6.1×10^{-7} M), but inhibited by verapamil (5.1 and 51.0×10^{-7} M).

5 The vascular relaxant effects of nitroglycerine and papaverine were inhibited in the presence of IBMX (4.4×10^{-5} M) or verapamil (5.1 and 51.0×10^{-7} M). Papaverine (6.1×10^{-7} M) also antagonized nitroglycerine-induced vascular relaxation.

6 The vasodilator effect of IBMX was greatly reduced in the presence of papaverine (6.1×10^{-7} M) or verapamil (5.1 and 51.0×10^{-7} M).

7 The vascular relaxant effect of verapamil was reduced proportionally by raising the extracellular Ca^{2+} concentration from 1.25 to 5.0 mM while those elicited by histamine, adenosine, isoprenaline, nitroglycerine, papaverine and IBMX were not modified by this procedure.

8 These results were taken as an indication that several vasodilators (e.g. histamine, adenosine, isoprenaline, nitroglycerine, papaverine and IBMX), but not a Ca^{2+} antagonist such as verapamil, produce a fraction of their vasodilator effects by promoting Ca^{2+} extrusion from and/or Ca^{2+} sequestration into the vascular smooth muscle cells, via a cyclic adenosine 3′,5′-monophosphate-dependent mechanism.

Introduction

In an isolated organ bath, the relaxation of vascular smooth muscle induced by vasodilators is likely to be the result of a decrease in the intracellular concentration of activator free Ca^{2+}. This final step in the mechanism of vascular relaxation may be brought about by various mechanisms: (1) interference with the entry of Ca^{2+} into the cells; (2) increased sequestration of Ca^{2+} in various intracellular sites; (3) decreased mobilization of Ca^{2+} from intracellular organelles or binding sites; (4) extrusion of Ca^{2+} from the cells. Evidence supporting the existence of such mechanisms has been reviewed recently (Bohr & Webb, 1978). The precise molecular mechanism by which vasodilators produce their effect has not yet been elucidated. However, current hypothesis suggest that adenosine cyclic 3′,5′-monophosphate (cyclic AMP) may be the intracellular mediator of several vasodilators (Triner, Nahas, Vulliemoz, Overweg, Verosky, Habif & Ngai, 1971; Andersson, 1972). The intracellular accumulation of cyclic AMP induced by drugs which activate adenylate cyclase or inhibit tissue phosphodiesterases, would contribute to Ca^{2+} sequestration in, and/or Ca^{2+} extrusion from, the smooth muscle cells (Andersson, 1972; Rasmussen, 1976). The precise molecular mechanism by which cyclic AMP may facilitate these two processes and contribute to vascular relaxation is still a matter of speculation. An interesting hypothesis holds that cyclic AMP would contribute to vascular relaxation by stimulating energy-requiring Ca^{2+} binding, and/or Ca^{2+} extruding, processes (Andersson, 1972; Bohr & Webb, 1978).

Recently, a protocol was designed which allowed precise measurement of vasodilator effects in the pre-

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sence of phosphodiesterase inhibitors (e.g. papaverine) or a Ca^{2+} antagonist (e.g. verapamil) (Gagnon, Regoli & Rioux, 1980). Using this approach, we observed that the vascular relaxant effects of glucagon and cyclic AMP in strips of rabbit renal artery moderately contracted with noradrenaline were potentiated by several phosphodiesterase inhibitors, inhibited by verapamil, but not affected by raising the extracellular Ca^{2+} concentration from 1.25 to 5.0 mM. As expected, the later intervention (e.g. increase in extracellular Ca^{2+} concentration) markedly inhibited the vasodilator effect of verapamil in the same preparation. Since the removal of extracellular Ca^{2+} with disodium edetate (Na_2EDTA), a metal chelating agent, provoked a vasodilator effect which was also blocked markedly by verapamil, we suggested that the mechanism by which verapamil inhibits the vascular relaxant effects of glucagon, cyclic AMP and Na_2EDTA results from its ability to interfere with the process of Ca^{2+} extrusion from the vascular smooth muscle cells and/or Ca^{2+} sequestration into intracellular sites. The vasodilator effects of glucagon, mimicked by cyclic AMP, was attributed to the facilitation of Ca^{2+} extrusion from the cells and/or Ca^{2+} uptake into intracellular sites via a cyclic AMP-dependent mechanism (Gagnon *et al.*, 1980). This interpretation being consistent with currently available models of vascular relaxation, we felt that it would be worthwhile to investigate the mechanisms of action of other vasodilators using the same experimental approach. The results described in this paper are consistent with the view that several vasodilators may produce their vascular effects by promoting the extrusion of Ca^{2+} from the vascular smooth muscle cells and/or Ca^{2+} uptake into intracellular sites, via a cyclic AMP-dependent mechanism.

Methods

General procedures

The experiments were performed on renal arteries derived from albino rabbits (1.2 to 1.5 kg) of either sex. The animals were killed by a blow on the neck and bled by cutting the carotid arteries. The renal arteries were taken out, freed from blood and fat and cut helically into strips 2 to 2.5 cm long and 1 to 1.5 mm wide. The tissues were suspended under a resting tension of 1 g in 40 ml organ baths containing a warm (37°C), oxygenated (95% O_2 : 5% CO_2) Krebs solution (Gagnon, Regoli & Rioux, 1978). Changes of tension of the tissues were measured isometrically by means of force displacement transducers (Grass FT03) coupled to a Grass polygraph (Model 79). The tissues were equilibrated for 90 min before starting the injections of drugs. Volume of drug injections varied between 0.1 and 0.4 ml.

Construction of dose-response curves

Control dose-response curves were obtained by applying cumulatively increasing concentrations of histamine, adenosine, isoprenaline, papaverine, IBMX, nitroglycerine or verapamil to strips of rabbit renal arteries kept contracted with a low concentration of noradrenaline (NA) (5.9 to 8.0×10^{-9} M) which increases the tension of the tissues by 0.8 to 1 g. After several washouts, the tissues were pretreated for 20 min with papaverine (6.1×10^{-7} M), IBMX (4.4×10^{-5} M) or verapamil (5.1 and 51.0×10^{-7} M). These drugs did not influence the basal tone of the tissues but higher concentrations of NA (see Table 1) had to be used in their presence in order to increase the tension of the tissues to the same level (0.8 to 1 g) as in their absence. When a stable contraction (plateau) was obtained, the injections of each vasodilator were repeated as above. The extent of the shift of the dose-response curve of each vasodilator provoked by papaverine, IBMX or verapamil was evaluated by dividing the ED_{50} values (concentration of vasodilator producing half-maximal relaxation) obtained in the presence and absence of these drugs. Each concentration of papaverine, IBMX or verapamil used to pretreat the tissues before repeating the injections of the various vasodilators was carefully selected on the basis of its ability to potentiate maximally (for papaverine and IBMX) or to inhibit the relaxant effect of glucagon in the same tissue (Gagnon *et al.*, 1980). The vasodilator effect of histamine was always measured in the presence of diphenhydramine (1.0×10^{-5} M) in order to eliminate its H_1-receptor-mediated contractile effect (Gagnon *et al.*, 1978).

Dose-response curves were also obtained with each vasodilator in tissues contracted with NA and pre-incubated in a low (1.25 mM), medium (2.5 mM) or relatively high (5.0 mM) Ca^{2+} containing Krebs solution.

Drugs

The following drugs were used: histamine dihydrochloride (Fisher), noradrenaline (NA), adenosine, papaverine hydrochloride, IBMX (Sigma), isoprenaline hydrochloride (Winthrop), nitroglycerine (Eli Lilly), diphenhydramine hydrochloride (Parke Davis), and verapamil (Knoll AG). NA was dissolved in saline HCl (0.01 N). Ascorbic acid was added to each dilution of NA. All other drugs were dissolved in saline (0.9% w/v NaCl). Concentrations of all drugs are expressed in mol/l of the salt except for nitroglycerine, IBMX, and adenosine (mol/l of the base).

Statistical significance was evaluated by Student's *t* test for paired or independent samples and *P* values of 0.05 or less were considered to be significant.

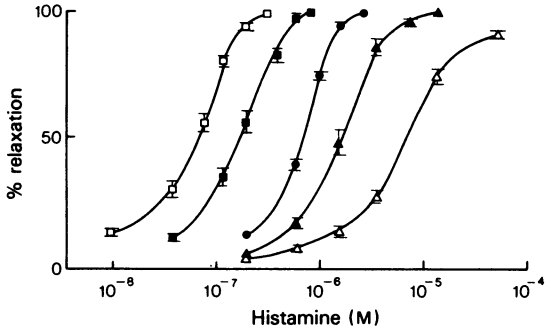

Figure 1 Dose-response curves for histamine as measured in strips of rabbit renal artery contracted with noradrenaline (NA) in the absence (control, ●) or presence of papaverine 6.1×10^{-7} M (■), 3-isobutyl-l-methylxanthine 4.4×10^{-5} M (□), or verapamil 5.1×10^{-7} (▲) and 5.1×10^{-6} M (△). Each point is the mean value; vertical lines show s.e. means. The number of individual determinations and the concentration of NA used to contract the tissues are given in Table 1.

Results

Effects of papaverine, 3-isobutyl-1-methylxanthine and verapamil on the vascular relaxant effect of several vasodilators

Histamine We have shown previously that papaverine (6.1×10^{-7} M) and IBMX (4.4×10^{-5} M) produced a maximal potentiation of the vasodilator effect of glucagon in strips of rabbit renal artery partially contracted by a low concentration of NA, while verapamil (5.1 and 51.0×10^{-7} M) inhibited dose-dependently the effect of glucagon in this tissue (Gagnon *et al.*, 1980). The results illustrated in Figure 1 indicate that the vascular relaxant effect of histamine is also markedly increased in the presence of papaverine (6.1×10^{-7} M) and IBMX (4.4×10^{-5} M), and inhibited in the presence of verapamil (5.1 and 51.0×10^{-7} M). The ED_{50} values of histamine and other vasodilators are summarized in Table 1. The dose-response curve for histamine is shifted to the left by a factor of 4.3 ± 0.5 in the presence of papaverine and 9.6 ± 0.7 in the presence of IBMX, but it remains parallel to the control curve. The extent of these shifts is very similar to that reported previously for glucagon (Gagnon *et al.*, 1980) and similar concentrations of papaverine and IBMX (Table 2).

The dose-response curve for histamine is shifted to the right dose-dependently in the presence of verapamil but it remains parallel to the control curve. The dose-ratios are equal to 2.4 ± 0.2 and 13.6 ± 2.2 in the presence of verapamil, 5.1×10^{-7} M and 5.1×10^{-6} M, respectively. Histamine appears to be more strongly inhibited by verapamil (5.1×10^{-6} M) than glucagon (Table 2). The maximum response for histamine is slightly depressed in the presence of verapamil (5.1×10^{-6} M).

Adenosine Figure 2 illustrates the dose-response curves for adenosine measured in the absence and presence of papaverine (6.1×10^{-7} M), IBMX (4.4×10^{-5} M) or verapamil (5.1 and 51.0×10^{-7} M). In the presence of papaverine, the dose-response curve to adenosine is shifted to the left by a factor of 5.3 ± 0.6, but it remains parallel to the control curve. On the other hand, the dose-response curve is shifted to the right when measured in the presence of IBMX or verapamil. Moreover, a substantial reduction of the maximum vasodilator effect of adenosine is observed in the presence of verapamil.

Isoprenaline The vasodilator effect of isoprenaline in strips of rabbit renal artery contracted moderately with NA is very small. The maximum response never exceeds 40% (curves not shown) even when measured in the presence of phentolamine in a tissue contracted with histamine (Gagnon, Regoli & Rioux, unpublished results). IBMX (4.4×10^{-5} M) potentiated by a factor of 7.4 ± 1.1 the vascular relaxant effect of isoprenaline without increasing its maximum response. In the presence of verapamil (5.1×10^{-6} M) the dose-response curve for isoprenaline is displaced to the right and the maximum response is reduced by 50%. The ED_{50} and dose-ratio values of isoprenaline are presented in Tables 1 and 2.

Nitroglycerine As shown in Figure 3, the dose-response curve for nitroglycerine is displaced to the right by a factor of 2.7 ± 0.3 in the presence of IBMX (4.4×10^{-5} M), and 3.8 ± 0.3 or 5.2 ± 0.4 in the presence of verapamil 5.1 and 51.0×10^{-7} M, respectively. There is no loss of parallelism. The ED_{50} and dose-ratio values are summarized in Tables 1 and 2, respectively. Papaverine (6.1×10^{-7} M) also inhibits the vasodilator effect of nitroglycerine (Table 1).

Papaverine The vascular relaxant effect of papaverine is inhibited in the presence of IBMX (4.4×10^{-5} M), and verapamil (5.1 and 51.0×10^{-7} M). The dose-response curve for papaverine is shifted to the right by a factor of 7.6 ± 1.8, 3.0 ± 0.3 or 5.1 ± 0.5 in the presence of IBMX or verapamil, 5.1 and 51.0×10^{-7} M, respectively (Figure 4, Tables 1 and 2). The maximum response to papaverine is not depressed by these 3 drugs.

3-Isobutyl-1-methylxanthine The dose-response curves for IBMX are shown in Figure 5. In the presence of papaverine (6.1×10^{-7} M), the dose-response curve for IBMX is displaced to the right by a factor of 2.1 ± 0.1 but there is no reduction in the maximum

Table 1 ED_{50} values of several vasodilators as determined in strips of rabbit renal artery contracted to a similar extent with noradrenaline (NA) in the absence (control) or presence (+) of the phosphodiesterase inhibitors, papaverine (PPV) or 3-isobutyl-1-methylxanthine (IBMX) or the calcium antagonist, verapamil (VRPM)

	ED_{50} values (mol/l) measured in the presence of:				
Vasodilators	NA (5.9×10^{-9} M)	NA (8.8×10^{-8} M) + PPV (6.1×10^{-7} M)	NA (2.0×10^{-7} M) + IBMX (4.4×10^{-5} M)	NA (7.0×10^{-8} M) + VRPM (5.1×10^{-7} M)	NA (4.4×10^{-7} M) + VRPM (5.1×10^{-6} M)
Histamine ($\times 10^{-7}$ M)	6.86 ± 0.24 (32)	1.75 ± 0.17* (10)	0.7 ± 0.05* (8)	16.2 ± 1.5* (11)	84.0 ± 12.0* (11)
Adenosine ($\times 10^{-6}$ M)	1.83 ± 0.19 (12)	0.35 ± 0.04* (8)	6.34 ± 0.59* (8)	4.52 ± 0.65* (11)	7.57 ± 2.1* (4)
Isoprenaline ($\times 10^{-7}$ M)	1.18 ± 0.04 (19)		0.17 ± 0.02* (8)	6.48 ± 0.86* (4)	64.7 ± 5.7* (7)
Nitroglycerine ($\times 10^{-9}$ M)	5.45 ± 0.26 (32)	15.4 ± 2.2* (6)	14.9 ± 2.2* (9)	21.1 ± 1.7* (12)	33.0 ± 3.9* (8)
Papaverine ($\times 10^{-7}$ M)	9.87 ± 0.59 (24)	—	64.9 ± 8.4 (8)	28.6 ± 1.4* (8)	42.7 ± 2.6* (8)
IBMX ($\times 10^{-6}$ M)	3.53 ± 0.26 (22)	5.94 ± 0.44* (8)	—	10.1 ± 0.8* (8)	13.4 ± 1.6* (6)
Verapamil ($\times 10^{-7}$ M)	1.52 ± 0.15 (8)	1.71 ± 0.26 (8)	—	11.3 ± 1.1* (4)	218·0 ± 16.0* (4)

The results are expressed as means ± s.e. mean. The number of individual determinations is given in parentheses below the ED_{50} values. The statistical significance was calculated by comparing respectively for each vasodilator the ED_{50} value obtained in the absence (control) and presence of PPV, IBMX or VRPM. ED_{50} values were derived from the dose-response curves shown in Figures 1, 2, 3, 4, and 5.
*$P < 0.001$.

effect or in the slope. Verapamil (5.1 and 51.0 × 10^{-7} M) also inhibits to varying extents the vasodilator effect of IBMX (Tables 1 and 2).

The influence of various extracellular Ca^{2+} concentrations upon the vasodilator effects of histamine, adenosine, isoprenaline, nitroglycerine, papaverine, 3-isobutyl-1-methylxanthine and verapamil

These experiments were designed to evaluate the possibility that some vasodilators behave as Ca^{2+} antagonists and exert their effect by interfering with the entry of Ca^{2+} into the vascular smooth muscle cells. Theoretically, compounds producing their vasodilator effects by the above mechanism should be less active in the presence of a high Ca^{2+} concentration and more active in the presence of a reduced Ca^{2+} concentration. Several observations from this (Gagnon *et al.*, 1980) and other laboratories (for a review, see Fleckenstein, 1977) support this interpretation for drugs such as verapamil.

The ED_{50} values of histamine, adenosine, isoprenaline, nitroglycerine, papaverine and IBMX are neither increased nor decreased following changes of the extracellular Ca^{2+} concentration from 1.25 to 5.0 mM. These results are summarized in Table 3. On the other hand, the ED_{50} values of verapamil increased significantly when the extracellular Ca^{2+} concentration was raised from 1.25 to 5.0 mM.

Discussion

The vascular relaxant effect of histamine in strips of rabbit renal artery moderately contracted with NA was shown previously to depend on the presence in the tissues of cimetidine-sensitive, H_2-receptors (Gagnon *et al.*, 1978). In this paper, we observed that the vascular relaxant effect of histamine in the same preparation is greatly potentiated by papaverine and IBMX, two potent phosphodiesterase inhibitors (Triner *et al.*, 1971; Wells, Wu, Baird & Hardman, 1975), and inhibited dose-dependently by verapamil, a well known inhibitor of Ca^{2+} influx into contractile organs (Fleckenstein, 1977). The potentiation of histamine-induced vascular relaxation by papaverine

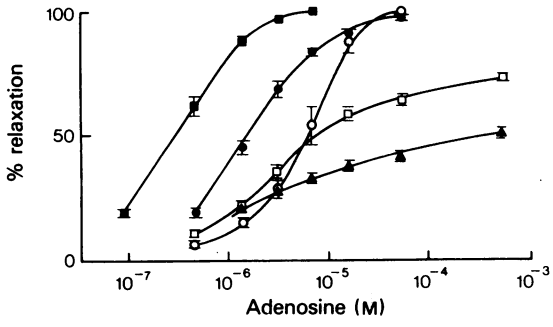

Figure 2 Dose-response curves for adenosine as measured in strips of rabbit renal artery contracted with noradrenaline (NA) in the absence (control, ●) or presence of papaverine 6.1 × 10^{-7} M (■), 3-isobutyl-1-methylxanthine 4.4 × 10^{-5} M (○) or verapamil 5.1 × 10^{-7} (□) and 5.1 × 10^{-6} M (▲). Each point is the mean value; vertical lines show s.e. means. The number of individual determinations and the concentrations of NA used to contract the tissues are given in Table 1.

Table 2 Dose-ratios for various vasodilators which were potentiated (+) or inhibited (−) by papaverine (PPV), 3-isobutyl-1-methylxanthine (IBMX) or verapamil (VRPM) in strips of rabbit renal artery contracted to a similar extent with noradrenaline (NA)[a]

	Dose-ratios measured in the presence of:			
Vasodilators	NA (8.8 × 10^{-8} M) + PPV (6.1 × 10^{-7} M)	NA (2.0 × 10^{-7} M) + IBMX (4.4 × 10^{-5} M)	NA (7.0 × 10^{-8} M) + VRPM (5.1 × 10^{-7} M)	NA (4.4 × 10^{-7} M) + VRPM (5.1 × 10^{-6} M)
Glucagon[b]	(+) 2.96	(+) 9.4	(−) 2.64	(−) 4.57
Histamine	(+) 4.3 ± 0.5	(+) 9.6 ± 0.7	(−) 2.4 ± 0.2	(−) 13.6 ± 2.2
Adenosine	(+) 5.3 ± 0.6	(−) 3.8 ± 0.4	(−) 2.5 ± 0.3	(−) 4.1 ± 0.9
Isoprenaline	—	(+) 7.4 ± 1.1	(−) 6.0 ± 0.6	(−) 58.5 ± 5.3
Nitroglycerine	(−) 3.5 ± 0.5	(−) 2.7 ± 0.3	(−) 3.8 ± 0.3	(−) 5.2 ± 0.4
Papaverine	—	(−) 7.6 ± 1.8	(−) 3.0 ± 0.3	(−) 5.1 ± 0.5
IBMX	(−) 2.1 ± 0.1	—	(−) 3.6 ± 0.1	(−) 4.3 ± 0.8
Verapamil	(−) 1.1 ± 0.2	(−) 1.1 ± 0.1	(−) 10.2 ± 1.2	(−) 195.8 ± 15.0

[a]The results are expressed as means ± s.e. mean. The number of individual determinations is as in Table 1. The dose-ratios were measured by dividing the ED_{50} values measured in the absence or presence of PPV, IBMX or VRPM.

[b]Data derived from Gagnon *et al.*, 1980.

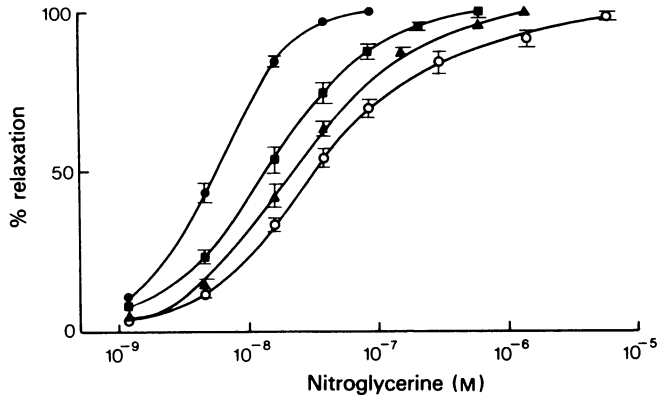

Figure 3 Dose-response curves for nitroglycerine as measured in strips of rabbit renal artery contracted with noradrenaline (NA) in the absence (control, ●) or presence of 3-isobutyl-l-methylxanthine 4.4×10^{-5} M (■) or verapamil 5.1×10^{-7} (▲) and 5.1×10^{-6} M (○). Each point is the mean value; vertical lines show s.e. means. The number of individual determinations and the concentrations of NA used to contract the tissues are given in Table 1.

and IBMX constitute indirect evidence for the participation of intracellular cyclic AMP in the vascular relaxant effect of histamine. The interaction of histamine with its specific receptors is likely to bring about the activation of vascular adenylate cyclase. The possibility that histamine acts as a phosphodiesterase inhibitor is rather unlikely since our results indicate that phosphodiesterase inhibitors antagonize each other (Figure 4 and 5). This is not surprising if one considers that these compounds presumably interact with the same enzyme. The extent of potentiation of histamine by papaverine and IBMX is very close to that reported previously for glucagon (Table 2) thus suggesting that histamine and glucagon produce their vascular relaxant effect through a common cyclic AMP-dependent mechanism.

Several lines of evidence suggest that verapamil produces its vasodilator and cardiodepressant effects by interfering with the entry of Ca^{2+} into vascular smooth muscle or cardiac muscle cells (for a review, see Fleckenstein, 1977). Whether or not verapamil has the ability to enter the cells and to interfere with the process of Ca^{2+} binding to, and/or sequestration into, intracellular elements is still unknown. In any case, the blockade of intracellular Ca^{2+} sequestration is expected to promote the cytoplasmic accumulation of free Ca^{2+} and to favour contraction rather than relaxation. Recently, we described the inhibitory action of verapamil toward glucagon and cyclic AMP-induced vascular relaxation (Gagnon *et al.*, 1980). This unexpected finding led us to suggest that verapamil interferes with Ca^{2+} extrusion from and/or Ca^{2+} sequestration into the smooth muscle cells and through these mechanisms, inhibits drug-induced vasodilator effects.

The blockade of histamine-induced vascular relaxant effect by verapamil suggest that histamine, like glucagon and cyclic AMP (Gagnon *et al.*, 1980), promotes Ca^{2+} extrusion from and/or Ca^{2+} sequestration into the vascular smooth muscle cells. Histamine is unlikely to interfere with the entry of Ca^{2+} into the cells because, in contrast to verapamil, its vasodilator effect is not antagonized by Ca^{2+} (Table 3).

The mechanism by which exogenous adenosine provokes the relaxation of isolated vascular tissues is not known. However, evidence suggests that adenosine interacts with specific receptors presumably located at the cell membrane level (Verhaeghe, 1978). The participation of intracellular cyclic AMP in the vascular relaxant effect of adenosine is still debated (Verhaeghe, 1978). Some results have indicated that adenosine causes a small increase in cyclic AMP level in resting arterial strips when an extremely high concentration of adenosine is used (Herlihy, Bockman, Berne & Rubio, 1976) while others demonstrated no increase at all (Verhaeghe, 1977). In this paper, we have shown that the vasodilator effect of adenosine was markedly potentiated by papaverine but inhibited by IBMX. The potentiation of the effect of adenosine by papaverine may be an indication that cyclic AMP mediates intracellularly the action of adenosine. However, an interference by papaverine with the uptake and/or degradation of adenosine in the tissues may also explain the potentiation of the adenosine effect by papaverine. This last hypothesis is consistent with other results showing the inhibition of adenosine uptake in vascular endothelial cells by papaverine (Pearson, Carleton, Hutchings & Gordon, 1978). The inhibitory effect of IBMX against adenosine adds further support to the view that several xanthine or xanthine derivatives compete with specific adenosine receptors at the cell membrane level (Toda, Hojo,

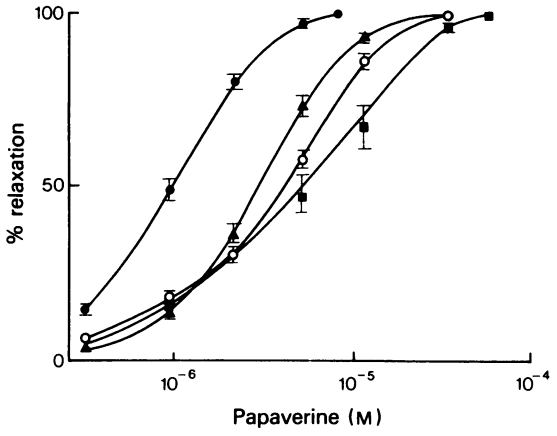

Figure 4 Dose-response curves for papaverine as measured in strips of rabbit renal artery contracted with noradrenaline (NA) in the absence (control, ●) or presence of 3-isobutyl-l-methylxanthine 4.4×10^{-5} M (■) or verapamil 5.1×10^{-7} (▲) and 5.1×10^{-6} M (○). Each point is the mean value; vertical lines show s.e. means. The number of individual determinations and the concentrations of NA used to contract the tissues are given in Table 1.

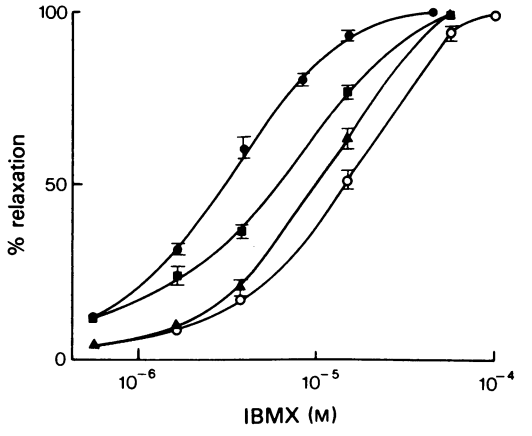

Figure 5 Dose-response curves for 3-isobutyl-l-methylxanthine (IBMX) as measured in strips of rabbit renal artery contracted with noradrenaline (NA) in the absence (control, ●) or presence of papaverine 6.1×10^{-7} M (■) or verapamil 5.1×10^{-7} (▲) and 5.1×10^{-6} M (○). Each point is the mean value; vertical lines show s.e. means. The number of individual determinations and the concentrations of NA used to contract the tissues are given in Table 1.

Sakae & Usui, 1975; Verhaeghe, 1977; 1978; Gagnon *et al.*, 1980). Adenosine is unlikely to act as a phosphodiesterase inhibitor because it does not potentiate glucagon or histamine (Gagnon, Regoli & Rioux, unpublished observations).

Adenosine has been claimed to relax depolarized coronary artery strips by blocking Ca^{2+} influx (Turnheim, Pittner, Kolassa & Kraupp, 1977). This conclusion was based on the ability of Ca^{2+} to inhibit the vascular relaxant effect of a relatively high concentration (10^{-4} M) of adenosine. Our results and those of Verhaeghe (1977) are not consistent with this interpretation. In fact, the vascular relaxant effect of adenosine in strips of rabbit renal artery partially contracted with NA was not affected by raising the extracellular Ca^{2+} concentration from 1.25 to 5.0 mM while, under the same conditions, the effect produced by verapamil was clearly antagonized (Table 3). The blockade of adenosine-induced vascular relaxation by verapamil was taken as an indication that adenosine

Table 3 ED_{50} values of several vasodilators as determined in strips of rabbit renal artery contracted with noradrenaline (7.3×10^{-9} M) in the presence of different extracellular Ca^{2+} concentrations[a]

	ED_{50} values measured in the presence of:		
Vasodilators	1.25 mM Ca^{2+}	2.5 mM Ca^{2+}	5.0 mM Ca^{2+}
Histamine ($\times 10^{-7}$ M)	7.07 ± 0.25	6.94 ± 0.36	6.90 ± 0.25 (12)
Adenosine ($\times 10^{-6}$ M)	1.01 ± 0.06	1.01 ± 0.05	1.15 ± 0.10 (6)
Isoprenaline ($\times 10^{-7}$ M)	1.26 ± 0.12	1.28 ± 0.08	1.19 ± 0.10 (8)
Nitroglycerine ($\times 10^{-9}$ M)	6.03 ± 0.66	6.60 ± 0.57	5.58 ± 0.62 (8)
Papaverine ($\times 10^{-7}$ M)	8.69 ± 0.61	10.53 ± 0.99	9.93 ± 0.92 (11)
IBMX ($\times 10^{-6}$ M)	3.66 ± 0.43	3.55 ± 0.41	3.59 ± 0.36 (7)
Verapamil ($\times 10^{-8}$ M)	5.72 ± 0.50**	19.0 ± 2.6	48.2 ± 7.3* (8)

[a]The results are expressed as means ± s.e. means. The number of individual determinations is given in parantheses at the right of the table. The statistical significance was evaluated by comparing the ED_{50} values obtained in 1.25 or 5·0 mM Ca^{2+} medium with those measured in 2.5 mM Ca^{2+} medium, for each compound.
*$P < 0.005$; **$P < 0.001$.

also exerts its vascular effect by promoting Ca^{2+} extrusion from, and/or Ca^{2+} sequestration into, the smooth muscle cells. The reason why the maximum vasodilator effect of adenosine, like that of cyclic AMP (Gagnon *et al.*, 1980) but unlike that of histamine and glucagon, was reduced by verapamil, is unknown.

The vascular relaxant effect of isoprenaline was potentiated by IBMX but inhibited by verapamil (Tables 1 and 2). However, isoprenaline-induced vascular relaxations were not modified by raising the extracellular Ca^{2+} concentration of the bathing solution from 1.25 to 5.0 mM. Taken all together, these results suggest that isoprenaline does not produce its vascular relaxant effect by blocking Ca^{2+} influx. More probably, isoprenaline acts by stimulating Ca^{2+} efflux from, and/or Ca^{2+} sequestration into, the cells through a cyclic AMP-dependent mechanism. Our suggestion that cyclic AMP may participate in the vascular relaxant effect of isoprenaline is consistent with the results of several workers (Triner *et al.*, 1971; Andersson, 1972). Papaverine was also found to potentiate the vascular relaxation induced by isoprenaline (Gagnon, Regoli & Rioux, unpublished observations).

Nitroglycerine behaves in our preparation as a phosphodiesterase inhibitor. It significantly potentiates the vascular relaxant effect of histamine and glucagon (Gagnon, Regoli & Rioux, unpublished results). Moreover, it was antagonized by phosphodiesterase inhibitors (IBMX and papaverine) which were found to potentiate the vascular action of glucagon and histamine (Table 2) and to antagonize each other (Figures 4 and 5, and Tables 1 and 2). The cross-inhibition between nitroglycerine, papaverine and IBMX is suggestive of a common site and/or mechanism of action for these three compounds. The ability of nitroglycerine to inhibit phosphodiesterase has been described previously (Andersson, 1972).

The vascular relaxant effects of nitroglycerine, papaverine and IBMX were not modified by varying the extracellular concentration of Ca^{2+} from 1.25 to 5.0 mM while those elicited by verapamil were inhibited (Table 3). This suggests that none of these three phosphodiesterase inhibitors interfere with the entry of Ca^{2+} into the vascular smooth muscle cells, at least in our experimental conditions. Recent studies have shown that papaverine inhibits Ca^{2+} uptake by K^+-depolarized taenia caecum of guinea-pigs (Takayanagi, Karasama & Kasuya, 1978) or by K^+-depolarized strips of rabbit main pulmonary artery (Thorens & Haeusler, 1979). The discrepancy between these results and ours may be explained by the different experimental procedures used (e.g. K^+ vs NA stimulation; different levels of tissue tension; different times of incubation of the tissues with papaverine). Our results do not exclude the possibility that under certain experimental conditions part of the vasodilator effect of high concentrations ($>10^{-5}$ M) of papaverine may be due to interference with the entry of Ca^{2+} into the vascular smooth muscle cells. In any case, papaverine is a relatively weak Ca^{2+} antagonist in comparison with drugs such as D600, nifedipine or verapamil (Schümann, Görlitz & Wagner, 1975).

The vasodilator effects of nitroglycerine, papaverine and IBMX were also inhibited to varying degrees by verapamil (Figures 4 and 5, Tables 1 and 2). This result was taken as an indication that the three phosphodiesterase inhibitors have the ability to promote Ca^{2+} extrusion from, and/or Ca^{2+} sequestration into, the vascular smooth muscle cells, most probably via a cyclic AMP-dependent mechanism (see, Introduction).

A technical point also needs to be discussed briefly. In the presence of papaverine, IBMX and verapamil, the concentration of NA required to raise the tension of the tissue by 0.8 to 1 g had to be increased since these drugs are vasodilators and interfere with the contractile effect of NA. Their effect was overcome and eliminated by increasing the concentration of the vasoconstrictor (i.e. physiological antagonism). Could the exposure of the tissue to high concentrations of NA be responsible for the inhibition of the vasodilator effects of histamine, adenosine, nitroglycerine, isoprenaline, papaverine and IBMX by verapamil? We do not think so mainly because relatively high concentrations of NA were also used to contract the tissues in the presence of phosphodiesterase inhibitors (e.g. papaverine and IBMX) and, in these conditions the vasodilator effects of histamine, adenosine, isoprenaline and glucagon were potentiated rather than inhibited.

In conclusion, the results described above are consistent with a model of vascular relaxation in which several vasodilators, except Ca^{2+} antagonists such as verapamil, produce a fraction of their vasodilator effect by promoting Ca^{2+} extrusion from, and/or Ca^{2+} sequestration into, the vascular smooth muscle cells, through a cyclic AMP-dependent mechanism. Further studies are obviously needed to substantiate this hypothesis. However, we believe that the pharmacological criteria applied in this study may be very useful for the classification of vasodilators. This study also raises the possibility that drugs of increasing clinical popularity such as Ca^{2+} antagonists (Fleckenstein, 1977; Rahwan, Piascik & Witiak, 1979) may exert some desirable and/or undesirable effects by counteracting the vasodilator effects of endogenous compounds (e.g. histamine, adenosine, prostaglandins, bradykinin, substance P and glucagon). Further studies are obviously needed before the several above hypotheses can be verified and substantiated.

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