Br. J. Pharmac. (1987), **91**, 439–446

pH-dependent stimulation by Ca^{2+} of prostacyclin synthesis in rat aortic rings: effects of drugs and inorganic ions

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1 Fresh rat aortic rings were incubated in HEPES-buffered salt solutions. Extracellular Ca^{2+} stimulated the production of prostacyclin (PGI_2), as determined by radioimmunoassay of its stable hydrolysis product 6-oxo-prostaglandin $F_{1\alpha}$. This action of Ca^{2+} was modified by H^+ over the pH range 8.0–6.5. Stimulation by calcium ionophore A23187 was not pH-dependent.

2 In parallel incubations of aortic rings with $^{45}Ca^{2+}$, followed by washing in the presence of La^{3+}, tissue uptake of $^{45}Ca^{2+}$ increased progressively as extracellular pH was increased from 6.5–8.0. Over this range intracellular pH, estimated by the distribution of [^{14}C]-dimethadione, varied from 5.9–7.4.

3 Stimulation by Ca^{2+} of PGI_2 synthesis was concentration-dependent over the range 0.7–20 mM. The maximum effect was an increase of approx. 4 fold.

4 Nifedipine, but not verapamil or diltiazem, inhibited Ca^{2+}-stimulated PGI_2 synthesis. A dihydropyridine compound that activates voltage-dependent Ca^{2+} channels, Bay K 8644, did not increase PGI_2 synthesis.

5 8-(N, N-diethylamino)-octyl-3,4,5 timethoxybenzoate (TMB-8), an antagonist of intracellular Ca^{2+} mobilisation, inhibited basal and Ca^{2+}-stimulated PGI_2 synthesis to a similar extent.

6 A solution containing 40 mM K^+ reduced Ca^{2+}-stimulated PGI_2 production. Mg^{2+} stimulated PGI_2 synthesis in a pH-dependent manner but was less potent than Ca^{2+}. Other divalent cations (Mn^{2+}, Ba^{2+} and Sr^{2+}), and La^{3+} had little or no effect on basal or Ca^{2+}-stimulated PGI_2 synthesis.

Introduction

Prostacyclin (PGI_2) is synthesized by vascular tissue in response to trauma (Bunting *et al.*, 1976). PGI_2 has potent actions on platelets and vascular smooth muscle (Moncada & Vane, 1979), and local PGI_2 synthesis by damaged blood vessels may be an important component of the vascular response to injury which is a process of major pathological significance (Ross, 1986). PGI_2 is not stored in tissues, and under physiological conditions hydrolyses spontaneously and rapidly to 6-oxo-prostaglandin (PG) $F_{1\alpha}$, which is inactive (Moncada & Vane, 1979). Of the processes determining PGI_2 concentration at a site of vascular injury, its synthesis is that most susceptible to physiological or pharmacological control. The present study of the influence of Ca^{2+} on PGI_2 synthesis was undertaken using freshly sliced rings of rat aorta as a model of vascular injury (Ritter *et al.*, 1982a).

PGI_2 synthesis is stimulated by calcium ionophore A23187 (Weksler *et al.*, 1978), and inhibited by 8 (N, N-diethylamino)-octyl-3,4,5 trimethoxybenzoate (TMB-8) (Brotherton & Hoak, 1982; Ritter, 1984), which is an antagonist of Ca^{2+} mobilisation from intracellular stores (Malagodi & Chiou, 1974). We previously showed that Ca^{2+} does not increase PGI_2 synthesis by fresh rat aortic rings in the absence of A23187 (Ritter *et al.*, 1982b), but these experiments were performed at a pH of 7 or less. We subsequently found that the action of a partially purified serum fraction on aortic PGI_2 synthesis was due to stimulation of PGI_2 synthesis by free extracellular Ca^{2+} at pH 8, the pH-dependence of this process accounting for the earlier negative observations (Taylor *et al.*, 1986). The object of the present study was to determine the concentration-effect relationships of Ca^{2+} and H^+ on aortic PGI_2 synthesis, and to investigate the mechanism of this interaction by measuring $^{45}Ca^{2+}$ uptake and determining the effects of drugs and inorganic ions known to influence Ca^{2+}-dependent processes.

Methods

Aortic ring incubations

Aortic rings were prepared by methods similar to those described previously (Bunting *et al.*, 1976; Ritter *et al.*, 1982a,b). Male CD rats (Charles River, Margate) 200–300 g were anaesthetized with ether. The aorta was removed rapidly and rinsed with Hanks' solution (Gibco, Uxbridge). It was cut into 1 mm rings with a McIlwain tissue chopper (Mickle Engineering Company, Guildford, Surrey). Rings were individually allocated to one of four groups so as to minimize differences between the groups. Each aorta yielded 4 groups of 12 rings. These were kept in Hanks' solution on ice for less than 30 min before incubation. This was started by adding tissue to incubation fluid (1 ml) at 37°C and performed for 60 min with constant shaking. Incubations were terminated by removing medium, which was stored at −20°C until assay for 6-oxo-$PGF_{1\alpha}$. Incubation fluid contained (mM) NaCl 120, KCl 4, glucose 5 and HEPES buffer 50, with additions of $CaCl_2$, $^{45}CaCl_2$ (0.5 μCi, 1.22 Ci mmol^{-1}), [^{14}C]-dimethadione (5, 5 dimethyl [2-^{14}C] oxazolidine-2, 4 dione) (DMO) (1 μCi, 43.2 mCi mmol^{-1}), [^{3}H]-sucrose (5 μCi, 10.1 Ci mmol^{-1}), other inorganic ions or drugs as indicated in the text. Radiochemicals were obtained from Amersham International, Amersham, (^{45}Ca) or New England Nuclear, Boston, Mass., U.S.A. In experiments with drugs, one group of rings from each aorta was incubated without drug or added Ca^{2+} (basal); the second with Ca^{2+} but without drug (Ca^{2+}-stimulated); the third with drug but no Ca^{2+} (to determine if the drug affected basal PGI_2 synthesis); and the fourth with drug and Ca^{2+} (to determine if the drug affected Ca^{2+}-stimulated PGI_2 synthesis).

Experiments on $^{45}Ca^{2+}$ uptake were performed by a method similar to that of van Breemen *et al.* (1972): after incubation in the presence of $^{45}Ca^{2+}$, tissue was washed in 2 × 10 min changes of incubation medium containing 2 mM $LaCl_3$ to displace extracellular bound Ca^{2+}. It was then incubated in 1 ml of Soluene (Packard instruments chemical division, Groningen, Netherlands) at 50°C for 2 h, and then overnight at room temperature until dissolution. The solution was decolourised by addition of propan-2-ol (200 μl) and hydrogen peroxide (30% w/v, 200 μl) and further incubation at 50°C for 2 h. This solution was mixed with 15 ml of 1:9 v/v 0.5 M HCl/Instagel (Packard instruments), and counted in a Packard tri-carb 2000CA liquid scintillation counter. Quenching, determined by an external gamma source, was similar in all samples.

Intracellular pH was estimated from the distribution of [^{14}C]-DMO and [^{3}H]-sucrose, using essentially the method of Waddell & Butler (1959). Total tissue water was measured by weighing before and after lyophilization. Drugs were purchased from Sigma (London) except methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay K 8644) which was a generous gift from Professor F. Hoffmeister (Bayer AG, Wuppertal, FRG). All other chemicals were Analar grade (BDH Chemicals, Poole, Dorset). Ionophore A23187 was dissolved in dimethylsulphoxide; TMB-8, nifedipine and Bay K 8644 were dissolved in ethanol; other drugs were dissolved in water. An equal volume of solvent was added to the control incubations in each case. Nifedipine and Bay K 8644 were protected from light and incubations with them performed in vials covered with aluminium foil.

Assay of 6-oxo-prostaglandin $F_{1\alpha}$

6-oxo-$PGF_{1\alpha}$ was determined by radioimmunoassay, using a previously described antibody (Hensby *et al.*, 1981; Orchard *et al.*, 1982), a generous gift from Dr L. Myatt (Institute of Obstetrics, Hammersmith Hospital, London). Briefly, assays were performed in triplicate on unextracted samples, using approximately 5 nCi (160 Ci mmol^{-1}) of [^{3}H]-6-oxo-$PGF_{1\alpha}$ (Amersham International, Amersham) per tube, and a final dilution of antiserum of 1:15,000. Unbound ligand was separated with activated charcoal; 50% displacement of tritiated ligand was caused by 64.9 ± 1.9 pg (mean ± s.e.mean) of standard 6-oxo-$PGF_{1\alpha}$. Standard 6-oxo-$PGF_{1\alpha}$ was a gift from Dr John Pike (Upjohn Co., Kalamazoo, MI, U.S.A.). Inter- and intra-assay variation were 14% and 7% respectively. Samples were diluted with phosphate buffered gelatin saline so that 0.1 ml caused 20–80% displacement of [^{3}H]-6-oxo-$PGF_{1\alpha}$. Triplicate assays were performed at different dilutions. Controls were performed that showed that, in the absence of unlabelled 6-oxo-$PGF_{1\alpha}$, relevant concentrations of the drugs, ions and solvents did not affect the binding of [^{3}H]-6-oxo-$PGF_{1\alpha}$ to antibody.

Analysis

Results are shown as mean ± s.e.mean, $n = 6$ unless stated otherwise. Comparisons were made by Student's paired t test and multi-factor analysis of variance on untransformed data and considered significant when $2P < 0.05$. The magnitude of drug effects was subsequently expressed as percentage change in basal or Ca^{2+}-stimulated 6-oxo-$PGF_{1\alpha}$ production.

Results

Aortic rings were incubated in the absence or presence of Ca^{2+} (20 mM) at pH 6.5, 7.0, 7.4 and 8.0. 6-oxo-

$PGF_{1\alpha}$ production is shown in Figure 1. There was a marked pH-dependence of the effect of Ca^{2+}, with marked stimulation occurring at pH 7.4 and 8.0 but not below pH 7.0. In contrast, A23187 (5×10^{-6} M, 2.5 mM Ca^{2+}) caused similar stimulation of 6-oxo-$PGF_{1\alpha}$ production at pH 7.0 (2.05 ± 0.06 fold) and pH 8.0 (1.92 ± 0.37 fold).

Aortic rings were incubated with $^{45}Ca^{2+}$ in the standard medium at pH 6.5, 7.0, 7.4, and 8.0. One group of the four groups of rings from each animal was incubated at each pH. $^{45}Ca^{2+}$ uptake is shown in Figure 2. There was a progressive increase in uptake as pH was increased.

Experiments in which aortic rings were incubated similarly, with [^{3}H]-sucrose and [^{14}C]-DMO, demonstrated an extracellular volume equivalent to 20.3% of tissue weight. Total water was 59.3% of tissue weight. The distribution of DMO indicated that intracellular pH reflected extracellular pH under the conditions of these incubations but was in general approx. 0.5 pH units below the extracellular pH (Table 1).

Incubations were also performed at different external Ca^{2+} concentrations to determine the dose-response relationship between external Ca^{2+} concentration and PGI_2 production. In each experiment one group of aortic rings served as control (no added Ca^{2+}), and the remaining 3 solutions contained different concentrations of Ca^{2+}. The effect of Ca^{2+} was expressed as the ratio of 6-oxo-$PGF_{1\alpha}$ produced in the presence to that produced in the absence of added Ca^{2+} ('fold stimulation'). Incubations were performed at extracellular pH 8.0 and pH 7.0. The results are shown in Figure 3. At pH 8.0 (pH 7.4 intracellular) there was a steep increase in 6-oxo-$PGF_{1\alpha}$ production

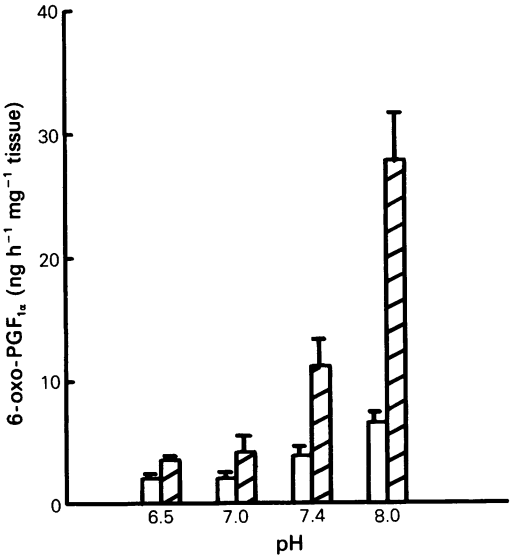

Figure 1 Stimulation of prostacyclin (PGI_2) synthesis by Ca^{2+} and its dependance on pH. Aortic rings were incubated in balanced salt solution at 37°C in the absence (open columns) or presence (hatched columns) of Ca^{2+} (20 mM). Stimulation occurred at pH 7.4 and pH 8.0 but was less marked below pH 7.0. There was significant interaction between pH and calcium stimulation ($2P < 0.0005$). At each pH, there was significant stimulation by calcium ($2P < 0.01$).

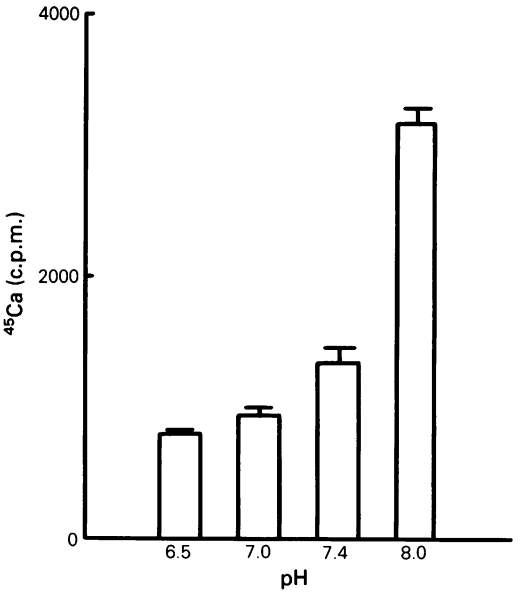

Figure 2 pH-dependence of $^{45}Ca^{2+}$ uptake by rat aorta. Aortic rings were incubated in the presence of Ca^{2+} (20 mM) and $^{45}CaCl_2$ (0.5 μCi) in balanced salt solution at 37°C buffered to the pH indicated. Tissue $^{45}Ca^{2+}$ was determined after washing with 2 mM $LaCl_3$. All samples were quenched to a similar extent. There was a significant increase in $^{45}Ca^{2+}$ uptake with increasing pH ($2P < 0.0005$, analysis of variance). $^{45}Ca^{2+}$ uptake at each pH was significantly greater than that at pH 6.5 ($2P < 0.05$) for each value.

Table 1 Extracellular and intracellular pH in rat aortic ring incubations

Extracellular pH	*Intracellular pH*[a]
6.5	5.9
7.0	6.6
7.4	6.9
8.0	7.4

[a] Intracellular pH determined from the distribution of [^{3}H]-sucrose and [^{14}C]-dimethadione in rat aortic rings after 1 h incubation at 37°C. Mean of 6 observations at each pH.

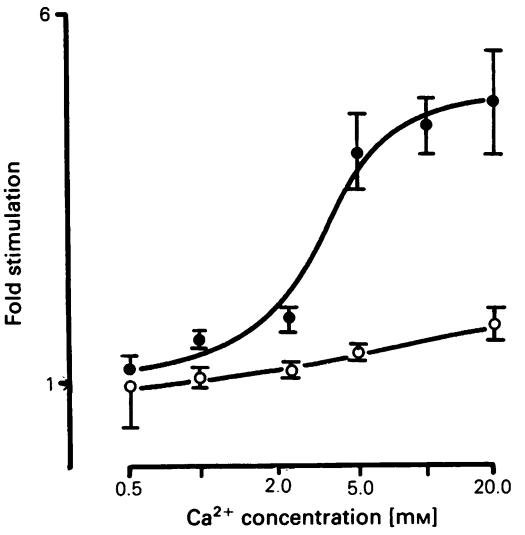

Figure 3 Stimulation by Ca^{2+} of prostacyclin (PGI_2) synthesis by rat aorta at pH 8 (●) and pH 7 (○). Aortic rings were incubated at 37°C in the presence of various concentrations of CA^{2+}. The effect of Ca^{2+} is expressed as the ratio between 6-oxo-$PGF_{1\alpha}$ produced in the presence of Ca^{2+} and that produced in the absence of Ca^{2+}.

between 2.5 and 5 mM Ca^{2+}, with the maximum at approximately 20 mM Ca^{2+}. In separate experiments no further increase of 6-oxo-$PGF_{1\alpha}$ occurred when the external Ca^{2+} concentration was further increased between 20 and 100 mM (data not shown). At extracellular pH 7.0 there was little stimulation of 6-oxo-$PGF_{1\alpha}$, even at 20 mM Ca^{2+}.

Effects of drugs that block voltage-dependent Ca^{2+} channels were determined at pH 8.0. Incubations were performed in the presence ('stimulated') or absence ('basal') of Ca^{2+} (20 mM), with or without Ca^{2+} antagonist. Results are shown in Table 2. Nifedipine (10^{-7} and 10^{-4} M) inhibited Ca^{2+}-stimulated but not basal 6-oxo-$PGF_{1\alpha}$ production. Verapamil and diltiazam had no significant effect, even at 10^{-4} M. A dihydropyridine analogue of nifedipine, Bay K 8644, that activates rather than inhibits voltage-dependent Ca^{2+} channels (Schramm *et al.*, 1983), was studied at 10^{-7}–10^{-4} M in the presence and absence of external Ca^{2+}. Concentrations of 2.5–20 mM Ca^{2+} were used to determine if Bay K 8644 altered the stimulation by

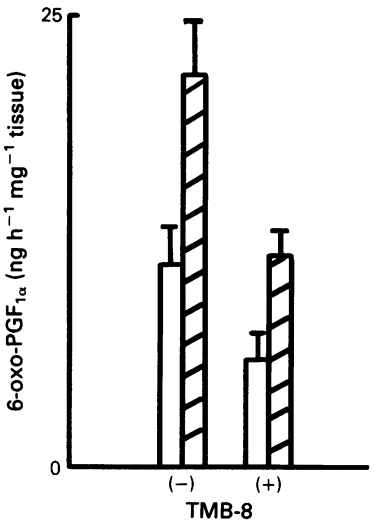

Figure 4 Effect of TMB-8 on prostacyclin (PGI_2) synthesis by rat aorta. Aortic rings were incubated in balanced salt solution at 37°C in the absence (open columns) or presence (hatched columns) of Ca^{2+} (20 mM), with (+) and without (−) 8-(N, N-diethylamino)-octyl-3,4,5 trimetroxybenzoate (TMB-8, 2×10^{-4} M). TMB-8 inhibited basal ($2P < 0.01$) and Ca^{2+}-stimulated ($2P < 0.01$) PGI_2 synthesis to a similar extent ($2P < 0.2$).

Table 2 Effect of Ca^{2+}-channel blocking drugs on prostacyclin (PGI_2) synthesis by rat aortic rings

Drug	*Concentration* (M)	*Effect of drug on basal* 6-oxo-$PFG_{1\alpha}$ *production* (% basal production[a])	*Effect of drug on* Ca^{2+} *– stimulated 6-oxo-*$PGF_{1\alpha}$ *production:* (%Ca^{2+} stimulated)[b]
Nifedipine	10^{-4}	110 ± 6.6 (6)	30.9 ± 6.7** (12)
	10^{-7}	—	74.8 ± 5.4* (6)
Verapamil	10^{-4}	89.5 ± 9.8 (6)	100.9 ± 7.2 (6)
Diltiazam	10^{-4}	150.6 ± 25.4 (6)	87.1 ± 16.9 (6)

[a] Basal 6-oxo-$PGF_{1\alpha}$ production in the presence of drug/basal 6-oxo-$PGF_{1\alpha}$ production in its absence × 100%.
[b] Ca^{2+}-stimulated 6-oxo-$PGF_{1\alpha}$ production in the presence of drug/Ca^{2+}-stimulated 6-oxo-$PGF_{1\alpha}$ production in its absence × 100%.
n is given in parentheses.
* $2P < 0.02$; ** $2P < 0.005$.

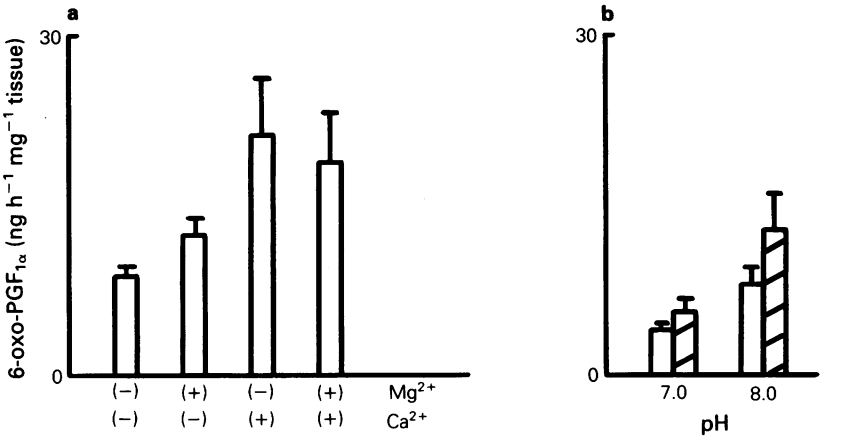

Figure 5 Effect of Mg^{2+} on prostacyclin (PGI_2) synthesis by rat aorta. (a) Aortic rings were incubated in balanced salt solution at 37°C at pH 8.0 in the absence and presence of Ca^{2+} (10 mM) and Mg^{2+} (10 mM) as indicated ($n = 7$). Mg^{2+} significantly ($2P < 0.02$) stimulated PGI_2 synthesis, but less potently than Ca^{2+}. The effect of Ca^{2+} and Mg^{2+} was not additive. (b) Rings were incubated at pH 7.0 and pH 8.0 in the absence (open columns) or presence (hatched columns) of Mg^{2+} (10 mM). Significant ($2P < 0.01$) stimulation of PGI_2 synthesis occurred at pH 8.0.

Ca^{2+} of PGI_2 synthesis, but there was no significant effect at any concentration (data not shown).

The effect of depolarization was determined in experiments in which aortic rings were incubated with and without added Ca^{2+} (10 mM) in the presence of a high concentration of K^+ (40 mM), or in the standard solution (4 mM K^+). The depolarizing solution had no effect on basal 6-oxo $PGF_{1\alpha}$ synthesis: 6-oxo-$PGF_{1\alpha}$ production in the presence of 4 mM K^+ was 6.94 ± 0.88 ng mg^{-1} h^{-1}, and in 40 mM K^+ was 6.98 ± 0.95 ng mg^{-1} h^{-1} ($n = 11$). However, 40 mM K^+ significantly reduced Ca^{2+}-stimulated 6-oxo-$PGF_{1\alpha}$ synthesis ($2P < 0.001$, $n = 11$), to 57.8% of the Ca^{2+} effect in the 4 mM K^+ solution.

The effect of TMB-8 on basal and Ca^{2+}-stimulated 6-oxo-$PGF_{1\alpha}$ production was determined in similar experiments. TMB-8 inhibited 6-oxo-$PGF_{1\alpha}$ production in the absence or presence of 20 mM Ca^{2+} to a similar extent, to $47.8 \pm 8.2\%$ and $48.6 \pm 4.0\%$ of control (mean ± s.e.mean) respectively (Figure 4).

The effects of several divalent cations (Mg^{2+}, Mn^{2+}, Ba^{2+} and Sr^{2+}), and of La^{3+} were determined. Four groups of rings from each aorta were used as before, and incubated in the standard salt solution at pH 8.0, alone (control), with 10 mM Ca^{2+}, with the cation under study, and with both Ca^{2+} and the other cation. Figure 5a shows the effect of Mg^{2+} (10 mM), which significantly stimulated 6-oxo-$PGF_{1\alpha}$ production, albeit less than an equal concentration of Ca^{2+}. The stimulation caused by Mg^{2+} was not additive with that caused by Ca^{2+}. In separate experiments ($n = 7$) this action of Mg^{2+} was found to be pH-dependent, being more marked at pH 8.0 than at pH 7.0 (Figure 5b). The other inorganic ions tested had no significant effect on basal or Ca^{2+}-stimulated 6-oxo-$PGF_{1\alpha}$ synthesis (Table 3).

Table 3 Effects of polyvalent cations on prostacyclin (PGI_2) synthesis by rat aorta

Ion	*Basal 6-oxo-$PGF_{1\alpha}$ production* (ng mg^{-1} h^{-1})		*Ca^{2+}-stimulated 6-oxo $PGF_{1\alpha}$ production* (ng mg^{-1} h^{-1})	
	(−)	(+)	(−)	(+)
$SrCl_2$ (10 mM)	5.49 ± 0.94	6.76 ± 0.57	11.09 ± 1.42	10.75 ± 0.34
$BaCl_2$ (10 mM)	7.00 ± 1.27	9.14 ± 0.89	15.33 ± 2.60	14.65 ± 3.02
$MnCl_2$ (10 mM)	3.49 ± 0.21	6.34 ± 0.11	8.49 ± 1.29	10.20 ± 0.68
$LaCl_3$ (2 mM)	7.55 ± 0.48	8.65 ± 0.42	13.56 ± 0.76	13.80 ± 1.36

6-oxo-$PGF_{1\alpha}$ production by rat aortic rings at 37°C in the presence (+) and absence (−) of polyvalent cations under basal and Ca^{2+}-stimulated (10 mM) conditions.

Discussion

We demonstrated previously that the PGI_2 stimulatory activity of a partially purified serum fraction was attributable to free Ca^{2+} (Taylor *et al.*, 1986). The present study, performed with balanced salt solutions of defined composition (rather than serum extracts), confirms that extracellular Ca^{2+} stimulates PGI_2 synthesis by rat aortic rings and that the effect is markedly pH-dependent. Stimulation occurs at physiological pH but is greater at pH 8.0 (Figure 1). In other experiments (not shown) we found no further increase in PGI_2 synthesis as pH was increased from pH 8.0 to pH 10.0. Significant stimulation of PGI_2 synthesis occurs at physiological Ca^{2+} concentration (Figure 3) but the steep part of the dose-response relation is at supraphysiological concentrations (2.5–5.0 mM).

It is, however, possible that extracellular Ca^{2+} is involved in PGI_2 responsiveness *in vivo*: thus infusions of calcium chloride into healthy human subjects have been reported to increase urinary 6-oxo-$PGF_{1\alpha}$ excretion, despite only a small increase in plasma Ca^{2+} concentration, and this effect is abolished by nifidepine (Nadler *et al*, 1986). The sensitivity to Ca^{2+} of PGI_2 synthesizing tissues *in vivo* may therefore be greater than in our *in vitro* system. If extracellular Ca^{2+} does stimulate PGI_2 synthesis at sites of vascular injury *in vivo*, tissue hypoxia (resulting in local acidosis) might terminate its action (cf. Figure 1), thereby contributing to the evolution of the pathological process.

The mechanism of the pH-dependence of this action of Ca^{2+} on PGI_2 synthesis is of considerable interest. The effect of the Ca^{2+} ionophore A23187 on PGI_2 synthesis (Weksler *et al.*, 1978) suggests that intracellular Ca^{2+} may control PGI_2 synthesis, and indeed phospholipase A_2 (Brokerhoff & Jensen, 1974), phospholipase C (Siess & Lapetina, 1983) and diacylglycerol lipase (Bell *et al.*, 1979) are Ca^{2+}-dependent. In the present study we confirmed that A23187 increases PGI_2 synthesis, and found additionally that the stimulation was similar at pH 7.0 and pH 8.0. The pH-dependence of the effect of Ca^{2+} on PGI_2 synthesis in the absence of A23187 could therefore be due to inhibition of cellular Ca^{2+} uptake by H^+. It had been reported, however, that in rabbit aorta, cellular Ca^{2+} influx is reduced as pH is increased from 6.5–8.0, while being markedly increased over a much wider pH range (2.5–10.5) (van Breemen *et al.*, 1972). We therefore used a method similar to that described by these authors to study $^{45}Ca^{2+}$ influx in rat aortic rings at the pH values at which H^+-dependent Ca^{2+} stimulation of PGI_2 synthesis occurred. We found (Figure 3) that Ca^{2+} influx rose progressively as pH was increased from 6.5–8.0. Interestingly, a similar inhibition by H^+ of Ca^{2+} entry in nerve and heart cells has been inferred from electrophysiological experiments (Vogel & Sperelakis, 1977; Spitzer, 1979). Part or all of the effect of H^+ on Ca^{2+}-stimulated PGI_2 synthesis could therefore be due to inhibition of cellular Ca^{2+} entry by H^+ in rat aortic rings. However, as expected, increasing extracellular pH also resulted in an increase in intracellular pH (Table 1). It is therefore also possible that the effect of pH could be at least partly due to an indirect action of extracellular H^+ concentration via intracellular pH.

Since the pH-dependence of the action of Ca^{2+} on PGI_2 synthesis may be due to an action of H^+ on cellular Ca^{2+} uptake (Figure 2), we studied drugs and ions known to inhibit various Ca^{2+} transport processes. Nifedipine inhibited Ca^{2+}-stimulated PGI_2 synthesis without affecting basal production (Table 2). However, this may not be due to its known action on voltage-dependent Ca^{2+} channels (Fleckenstein, 1977; Cauvin *et al.*, 1983), because (a) depolarization with KCl was found to reduce rather than increase Ca^{2+}-stimulated PGI_2 synthesis; (b) antagonists of voltage-sensitive Ca^{2+} channels not structurally related to nifedipine (diltiazem and verapamil) did not inhibit Ca^{2+}-stimulated PGI_2 synthesis (Table 2); (c) the structurally related dihydropyridine agonist Bay K 8644 did not augment Ca^{2+}-stimulated PGI_2 synthesis, and (d) inhibition by nifedipine was incomplete even at high concentration.

These properties differ from those of known voltage-sensitive Ca^{2+} channels (Nowycky *et al.*, 1985; Nilius *et al.*, 1985), and also differ from those of other preparations. Thus adrenaline-stimulated PGI_2 secretion by partially exhausted rat aortic rings is inhibited by verapamil (Jeremy *et al.*, 1985) and nifedipine (Jeremy *et al.*, 1986). Basal and agonist-stimulated PGI_2 synthesis by cultured mesothelial cells is inhibited by verapamil as well as nifedipine (van de Velde *et al.*, 1986), and diltiazem is reported to stimulate PGI_2 synthesis by human umbilical vein (Mehta *et al.*, 1986). Diltiazem may also have caused some stimulation at high concentration (10^{-4} M) in the present experiments (Table 2), although the difference did not reach significance ($2P > 0.05$). Nifedipine has no effect on basal or vasopressin-stimulated PGI_2 synthesis by cultured arterial smooth muscle cells, another preparation in which PGI_2 synthesis is dependent on extracellular Ca^{2+} (Hassid & Oudinet, 1986). PGI_2 synthesis by cultured porcine aortic endothelial cells is also dependent on extracellular Ca^{2+} but, as in our experiments, is not inhibited by verapamil (Whorton *et al.*, 1984). The mechanism and significance of the effect of nifedipine in the present experiments therefore remain uncertain. The inhibitory effect of K^+, which was unexpected, could however represent inactivation of a voltage-dependent Ca^{2+}-transport mechanism sensitive to nifedipine but not to the other drugs studied.

TMB-8, an inhibitor of intracellular Ca^{2+} mobilisa-

tion (Malagodi & Chiou, 1974), inhibited basal PGI_2 synthesis by rat aortic rings as described previously (Ritter, 1984). However, it also inhibited Ca^{2+}-stimulated PGI_2 synthesis to a similar extent (Figure 4). This observation is at odds with the explanation of Ca^{2+}-stimulated PGI_2 synthesis being simply due to cellular influx of Ca^{2+} across the plasma membrane. It is possible that Ca^{2+} entry triggers a further increase in cytoplasmic Ca^{2+} as a result of Ca^{2+}-stimulated Ca^{2+} release from intracellular organelles (Frank, 1980; Fabiato 1983). Such a process might be inhibited by TMB-8. Alternatively it is possible that TMB-8 has additional actions on PGI_2 synthesis independent of its effect on intracellular Ca^{2+} mobilisation (Oudinet & Hassid, 1984).

Several inorganic ions mimic or block the actions of Ca^{2+} (Kohlhardt *et al.*, 1973; Akaike *et al.*, 1978). Unexpectedly, Mg^{2+} caused pH-dependent stimulation of PGI_2 synthesis similar to, but less potent than, that caused by Ca^{2+} (Figure 5). This result must be interpreted cautiously since tissues contain extracellular bound Ca^{2+} (van Breemen *et al.*, 1972; Brading & Widdicombe, 1977) which may be displaced by extracellular Mg^{2+}, and it is therefore possible that the effect is due to release of bound Ca^{2+}. The stimulation of PGI_2 synthesis caused by Mg^{2+} was not additive with that of Ca^{2+} (Figure 5), suggesting that Mg^{2+} might have a dual action, displacing extracellular bound Ca^{2+} while simultaneously partially inhibiting its uptake across the plasma membrane. The other divalent ions studied, and also La^{3+}, had no significant effect on either basal or Ca^{2+}-stimulated PGI_2 synthesis (Table 3). The pharmacological properties of H^+-dependent Ca^{2+} stimulation of PGI_2 synthesis by rat aorta thus differ from those of known Ca^{2+} uptake processes. We are at present investigating the possibility of an intracellular interaction between H^+ and Ca^{2+} on PGI_2 synthesis.

This work was supported by the Medical Research Council. We thank Miss Bernadette Edinborough for excellent secretarial assistance.

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(Received January 15, 1987.
Revised February 24, 1987.
Accepted February 25, 1987).