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ACTIONS OF PROSTAGLANDIN $F_{2\alpha}$ AND NORADRENALINE ON CALCIUM EXCHANGE AND CONTRACTION IN RAT MESENTERIC ARTERIES AND THEIR SENSITIVITY TO CALCIUM ENTRY BLOCKERS

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1 The actions of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and noradrenaline on contraction and ^{45}Ca exchange have been studied in rat mesenteric arteries.

2 $PGF_{2\alpha}$ and noradrenaline contracted rat isolated mesenteric artery preparations to about the same extent. The $PGF_{2\alpha}$-stimulated contractions, unlike those produced by noradrenaline, were completely inhibited in calcium-free physiological solution.

3 The calcium entry blocking drugs, cinnarizine and flunarizine, had little effect on the resting exchange of calcium in the arterial smooth muscle, but inhibited $PGF_{2\alpha}$-stimulated contractions and ^{45}Ca uptake to a similar extent.

4 Flunarizine was about 7 fold more potent as an inhibitor of noradrenaline- than of $PGF_{2\alpha}$-mediated contraction and ^{45}Ca uptake and this ratio was about 50 for cinnarizine.

5 EGTA (1.25 mM) produced a relaxation of noradrenaline and $PGF_{2\alpha}$-induced maximal contractions. Measured over the first 2 min of EGTA contact, the rate of relaxation was much faster in noradrenaline than in $PGF_{2\alpha}$-stimulated preparations.

6 Turnover of cellular calcium (influx plus efflux) during the first 2 min of noradrenaline contact was much greater than that produced by $PGF_{2\alpha}$, largely due to a greater effect of noradrenaline on calcium efflux.

7 The results suggest that $PGF_{2\alpha}$- but not noradrenaline-induced contractions are entirely dependent on the influx of extracellular calcium and that the agonists may stimulate calcium gating mechanisms differently.

Introduction

It is generally accepted that contraction is initiated in vascular smooth muscle cells when the internal free calcium concentration rises above 0.1 μM (Bolton, 1979) and this can be achieved by the entry of extracellular calcium into the cell or by the mobilization of an intracellular calcium pool, as demonstrated by the persistence of contractile responses in the absence of external calcium (Godfraind & Kaba, 1969; Keatinge, 1972; Bohr, 1973; Godfraind, Kaba & Rojas, 1973; Karaki, Kubota & Urakawa, 1979; Bülbring, 1979; van Breemen & Siegel, 1980). This indirect evidence has been confirmed in studies using ^{45}Ca, showing that catecholamines increase the cell membrane permeability to extracellular ^{45}Ca and release ^{45}Ca stored in the cell (Godfraind, 1976; Deth & Lynch, 1981).

Catecholamines are able to utilize both of these calcium pools in most vascular smooth muscles studied (Godfraind & Kaba, 1969; Keatinge, 1972; Bohr, 1973; Godfraind *et al.*, 1973; Karaki *et al.*, 1979 but see van Breemen & Siegel, 1980), the release of intracellular calcium apparently being sufficient to produce nearly 50% of the maximal response in rat aorta (Godfraind & Dieu, 1981). The release of intracellular calcium produces a fast non-sustained contraction and calcium influx produces a sustained contraction (Godfraind & Kaba, 1969; 1972; Bolton, 1979).

The ability of prostaglandins to mobilize different calcium pools to produce contraction seems to be variable (see for example Levy, 1973; 1980; Altura & Altura, 1976; Mikhelson & Andersson, 1978) but whether the variation is due to species or tissue differences is not clear.

The purpose of this study was to analyse the relationship between calcium turnover and contraction stimulated by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and noradrenaline in rat mesenteric arteries. The calcium entry

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blocker, cinnarizine, and its bis-fluorophenyl derivative, flunarizine, which inhibit contractions produced by noradrenaline and depolarization by blockade of calcium entry channels (Godfraind, 1974; 1979; Godfraind & Dieu, 1981), were used to differentiate the part played in $PGF_{2\alpha}$-induced contractions by extracellular and intracellular calcium pools.

The results suggest that the actions of $PGF_{2\alpha}$ and noradrenaline on calcium fluxes are different and that the receptor-response coupling processes for these two compounds are not indentical.

A brief account of some of these results has been presented to the British Pharmacological Society (Godfraind & Miller, 1981).

Methods

Ten to fifteen week old male Wistar rats (weighing 240–350 g) were killed by decapitation. The superior mesenteric artery was removed, cleaned of all loosely adherent tissue and cut spirally according to the method of Furchgott (1960). Preparations were suspended in a 20 ml organ bath containing physiological solution (mM: NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2 under a tension of 2 g. Ca^+-free solution was prepared by omission of calcium.

Contractile responses were measured with an isometric transducer coupled to a potentiometric pen recorder. After an equilibration period of 60 min the preparations were contracted maximally in a depolarizing medium (mM: NaCl 17, KCl 100, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) (Godfraind & Kaba, 1969). After washing in physiological solution several times and a further 60 min equilibration period, cumulative concentration-effect curves for $PGF_{2\alpha}$ (0.4 to 33.3 μM) or noradrenaline (0.001 to 10 μM) were obtained by increasing the bath concentration in successive steps. After washing and when baseline tension had been regained, cinnarizine 0.1 to 10 μM or flunarizine 0.01 to 10 μM final bath concentration, was added and left in contact with the tissue for 90 min to attain equilibrium (Godfraind & Morel, 1981) after which time a second cumulative concentration-effect curve was obtained. On occasions preparations were then equilibrated with a second larger concentration of cinnarizine or flunarizine and a third cumulative concentration-effect curve obtained. Control experiments were performed using the same protocol but in the absence of cinnarizine or flunarizine. In other experiments single maximal contractions produced by $PGF_{2\alpha}$ (33.3 μM) or noradrenaline (10 μM), or by the depolarizing medium were used in place of cumulative additions of $PGF_{2\alpha}$. The effect of 1, 2, bis, 2 aminoethoxyethane-NNN'N'-tetracetic acid (EGTA) on these maximal contractions was studied by introducing it into the organ bath at a final concentration of 1.25 mM. When the EGTA-induced relaxation was complete the preparations were washed at least 12 times over a 120 min period before a second contraction was induced. In these experiments only depolarization and either noradrenaline or $PGF_{2\alpha}$ were used in any one preparation in order to avoid any modifying effect of $PGF_{2\alpha}$ on noradrenaline-induced contractions (Bolton, 1979).

Measurement of ^{45}Ca influx and efflux

The net rate of uptake of calcium into the smooth muscle cells of the rat mesenteric artery stimulated by $PGF_{2\alpha}$ and noradrenaline was estimated by measuring the increase in ^{45}Ca content of the smooth muscle produced by these agonists during exposure of the tissue to a ^{45}Ca containing physiological solution. Lanthanum, which has been shown to displace extracellular calcium while having little or no effect on the intracellular content of calcium under certain conditions (Mayer, van Breemen & Casteels, 1972; van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973; Godfraind, 1974; 1976) was used to remove the relatively large amount of ^{45}Ca in the extracellular space which would otherwise interfere with the determination of cellular ^{45}Ca content, by the method of Godfraind (1976).

The arteries were cut open longitudinally to form flat strips weighing about 4–6 mg and equilibrated for at least 60 min in physiological solution (mM: NaCl 122, KCl 5.9, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25, glucose 11) maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2. After a preincubation period of 90 min in physiological solution containing cinnarizine (1 to 10 μM) or flunarizine (0.01 to 10 μM), the artery strips were further incubated for 5 min in 6 ml of physiological solution containing ^{45}Ca (1 μCi/ml) as well as cinnarizine or flunarizine, then for another 2 min in the same solution with the addition of $PGF_{2\alpha}$ or noradrenaline. Thereafter, the preparations were washed for 5 min in 500 ml of a La^{3+} solution (mM: NaCl 122, KCl 5.9, $MgCl_2$ 1.25, $LaCl_3$ 50, glucose 11, Tris maleate 15 (pH 6.8)) to remove extracellular Ca^{2+} from the tissue. Parallel control experiments were always performed at the same time, an appropriate volume of vehicle replacing the $PGF_{2\alpha}$ or noradrenaline. After the La^{3+} wash, the artery strips were placed between two sheets of filter paper and pressed three times with a roller weighing 350 g. Each strip was weighed, dissolved in 0.1 ml of a solution composed of equal parts of perchloric acid (37% w/v) and

H_2O_2 (30 vol) by heating for 15 min at 75°C. After cooling, 5 ml of Aqualuma (Lumac) was added and the radioactivity of the samples counted in a liquid scintillation counter as usual, with appropriate controls. The results of each determination have been converted to the apparent tissue content of Ca (mmol/kg wet wt).

$$= \frac{\text{d/min in muscle}}{\text{wet wt (kg)}} \times \frac{\text{mmol Ca/l medium}}{\text{d min}^{-1}\,\text{l}^{-1}\,\text{medium}}.$$

$PGF_{2\alpha}$- and noradrenaline-dependent ^{45}Ca efflux was estimated in artery strips after they had been preincubated in ^{45}Ca (2 μCi/ml) containing physiological solution for 120 min. Tissues were then rinsed for 5 min in non-radioactive physiological solution before being transferred to non-radioactive solution containing either noradrenaline or $PGF_{2\alpha}$ in appropriate concentrations for a further 2 min, after which they were placed in lanthanum containing solution and treated as described for the ^{45}Ca influx experiments. Control preparations were rinsed in non-radioactive solution for 7 min. In all ^{45}Ca efflux experiments, equal numbers of control and agonist-treated preparations were always processed at the same time.

Drugs

Cinnarizine and flunarizine (Janssen Pharmaceutica) were dissolved in an aqueous solution of 100 mM tartaric acid (pH 3.1) to a concentration of 1 mM and further diluted as required with distilled water. $PGF_{2\alpha}$ tromethamine salt (Upjohn) was dissolved in absolute ethanol as a stock solution of 10 mg/ml and diluted in distilled water before use. Noradrenaline bitartrate (Flucker) was dissolved in distilled water containing 7.9 mM Na_2SO_3 and 34 mM HCl as a stock solution of 0.1 mM and diluted in 0.9% w/v NaCl solution (saline) before use. EGTA (Sigma) was dissolved in distilled water and buffered with tris-(hydroxymethyl)-aminomethane to pH 7.4. ^{45}Ca (specific activity approx 2 mCi/μmol) was obtained from the Radiochemical Centre, Amersham. All drug concentrations are expressed in terms of the base.

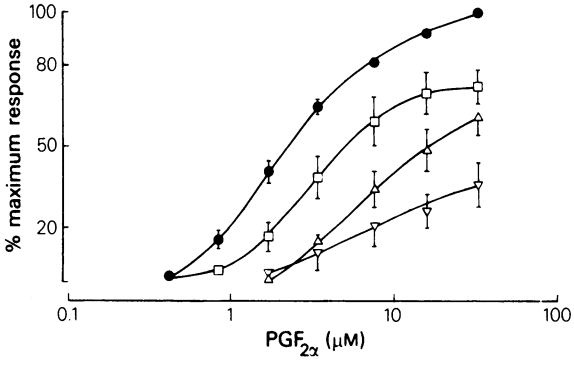

Figure 1 Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-stimulated concentration-effect curves in rat mesenteric arteries in the absence (●) and presence of cinnarizine 0.1 μM (□), 1 μM (△), and 10 μM (▽). Each curve is the mean of at least 4 preparations. Vertical bars represent s.e.mean.

Statistical analysis

The data are expressed as means ± s.e.mean. In the case of experiments involving ^{45}Ca, the number of observations (n) applies to both control and non-control groups. Tests of significance have been made using Student's t test, or paired t test where possible, P values smaller than 0.05 being considered significant. A least squares linear regression analysis has been used to fit straight lines to data where appropriate. The concentration of cinnarizine or flunarizine producing a 50% inhibition of the maximal contractile response (IC_{50}) or of the maximal $PGF_{2\alpha}$-dependent ^{45}Ca influx (I_{50}) and their s.e. were estimated from concentration-effect curves.

Results

Measurement of contractile responses

Reproducible cumulative concentration-effect curves to $PGF_{2\alpha}$ and noradrenaline could be obtained repeatly when separated by 90 min.

The maximum contraction of the artery when stimulated by $PGF_{2\alpha}$ (33.3 μM) was 156.4 ± 8.9% (n = 18) of that produced by the standard depolarizing solution. In arteries taken from a similar group of rats, noradrenaline (10 μM) produced a maximum contraction of 140.1 ± 7.4% (n = 19) of that produced by depolarization. These maximal contractions are not significantly different ($0.1 < P < 0.2$).

In two experiments $PGF_{2\alpha}$ at concentrations up to 33.3 μM failed completely to contract mesenteric artery preparations previously washed in a Ca^{2+}-free physiological solution for 10 min. These arteries still produced a 10 to 15% contraction in response to noradrenaline 10 μM. Both cinnarizine (0.1 to 10 μM) and flunarizine (0.01 to 1 μM) antagonized $PGF_{2\alpha}$-induced responses in a non-competitive manner producing a dose-related depression of cumulative concentration-effect curves (Figures 1 and 2). At concentrations of inhibitors greater than 0.1 μM there was also a small shift of the curves to the right. Flunarizine 10 μM completely inhibited contractile responses to $PGF_{2\alpha}$ at concentrations up to 33.3 μM (Figure 2).

The concentrations of cinnarizine and flunarizine producing a 50% depression of the maximal response

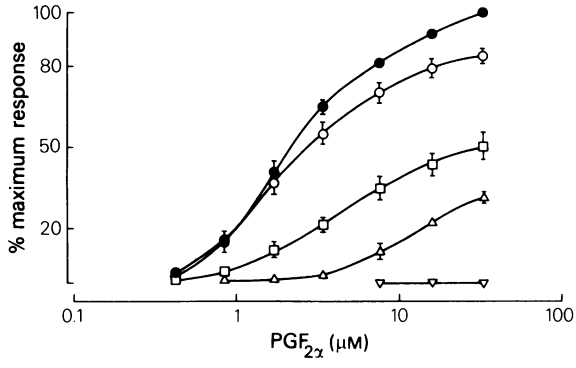

Figure 2 Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-stimulated concentration-effect curves in rat mesenteric arteries in the absence (●) ($n=17$) and presence of flunarizine 0.01 M (○) ($n=4$), 0.1 M (□) ($n=4$), 1 μM (△) ($n=3$) and 10 μM (▽) ($n=2$). Vertical bars represent s.e.mean.

to $PGF_{2\alpha}$ (IC_{50} values) were calculated to be 3.3 ± 0.3 μM and 0.14 ± 0.03 μM respectively and the IC_{50} value for cinnarizine depression of responses to noradrenaline was 0.06 ± 0.01 μM (Table 1).

Single applications of $PGF_{2\alpha}$ (33.3 μM) to mesenteric artery strips produced contractions which reached a maximum after about 10 min. The initial rate of increase in tension produced by $PGF_{2\alpha}$ was much lower than that produced by noradrenaline (Figure 3) but after a 2 min agonist contact time similar magnitudes of contraction were achieved, being $70.4 \pm 2.0\%$ of maximum ($n=11$) for noradrenaline and $72.6 \pm 3.0\%$ ($n=8$) for $PGF_{2\alpha}$. After 15 min contact, $PGF_{2\alpha}$-induced contractions began to decline slowly, a small significant relaxation usually becoming apparent after 25 min (Figure 4). A second single application of $PGF_{2\alpha}$ to the same preparation after 90 min incubation in flunarizine 0.1 μM produced a contraction that was depressed in magnitude but reached a plateau after about 15 min which was maintained for at least the next 15 min (Figure 4). Confirming the observations of Godfraind & Dieu (1981), single applications of noradrenaline 10 μM or K^+ depolarization of the mesenteric artery also produced sustained contractions. After 90 min incubation in flunarizine 0.03 μM, noradrenaline, like $PGF_{2\alpha}$ still produced a sustained although depressed contraction, but K^+ depolarization produced a response which, unlike that produced by noradrenaline and $PGF_{2\alpha}$, displayed a time-dependent decrease in tension to a secondary lower plateau in about 10–15 min which was maintained for at least the next 20 min (Figure 4).

The addition of EGTA (1.25 mM final bath concentration) during the sustained phase of maximal contraction induced by $PGF_{2\alpha}$ or noradrenaline produced a prompt reduction in the tension of the artery. Two min after addition of EGTA the noradrenaline-induced contraction had relaxed to $20.6 \pm 5.2\%$ ($n=5$) of its maximum while the $PGF_{2\alpha}$-induced contraction relaxed to $58.9 \pm 6.5\%$ ($n=6$) of its maximum. These initial rates of relaxation are significantly different ($0.001 < P < 0.01$).

Measurement of ^{45}Ca influx and efflux

The lanthanum-resistant ^{45}Ca content of arteries, measured after 2 min stimulation in concentrations of $PGF_{2\alpha}$ and noradrenaline that produce maximal contractions, were not statistically different, nor was the ^{45}Ca content of control arteries incubated in ^{45}Ca containing physiological solution (Table 2). The uptake of ^{45}Ca was dose-dependently stimulated by $PGF_{2\alpha}$, a concentration of 2.1 μM (the EC_{50} concentration) increased ^{45}Ca uptake by 17.7 ± 2.0 μmol Ca/kg ($n=8$) over that of controls while 33.3 μM stimulated the uptake of approx. 41.2 μmol Ca/kg ($n=6$).

Efflux of ^{45}Ca was significantly greater than that from control arteries after 2 min in the presence of noradrenaline (10 μM) or $PGF_{2\alpha}$ (33.3 μM), the efflux being approx. 60.1 ($n=8$) and approx. 31.1 ($n=8$) μmol Ca/kg respectively ($0.001 < P < 0.01$) (Table 2).

^{45}Ca influx measured in arteries incubated in the presence of cinnarizine and flunarizine at concentrations varying from 0.01 to 1 μM was not significantly different from influx measured in control preparations. When a concentration of 10 μM was used, there was a slight reduction of this uptake, ^{45}Ca content

Table 1 Concentrations of cinnarizine and flunarizine producing 50% inhibition of the contractile response (IC_{50}) and ^{45}Ca influx (I_{50}) in rat mesenteric arteries after a preincubation of 90 min

	IC_{50} (μM) *Contraction*		I_{50} (μM) *^{45}Ca Influx*	
	Cinnarizine	*Flunarizine*	*Cinnarizine*	*Flunarizine*
$PGF_{2\alpha}$ (33.3 μM)	3.3 ±0.3	0.14±0.03	2.9±0.3	0.13±0.01
Noradrenaline (10 μM)	0.06±0.01	0.02±0.01*		0.05*

Values are given ± s.e.mean. They are calculated by regression analysis.
*Values from Godfraind & Dieu (1981).

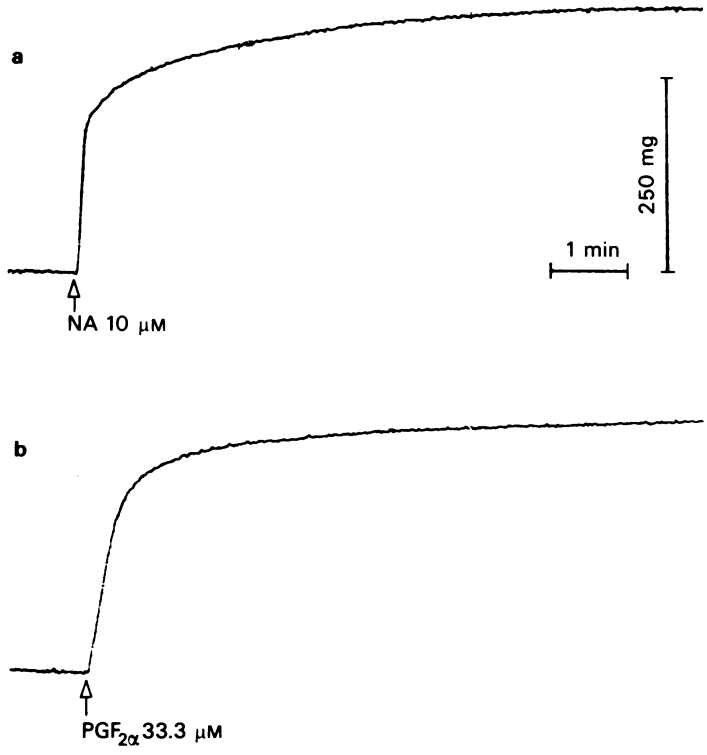

Figure 3 Time-contraction responses stimulated by noradrenaline 10 μM (a) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 33.3 μM (b) in the same rat mesenteric artery preparation, showing characteristic differences in the initial rate of increase of contractile force. A 60 min rest period was allowed between the two responses.

measured after 7 min being equal to 63.7 ± 3.7 μmol Ca/kg and 65.2 ± 3.4 μmol Ca/kg for cinnarizine and flunarizine respectively, as compared to 74.8 ± 2.3 μmol Ca/kg ($n = 12$) for control preparations. The $PGF_{2\alpha}$-dependent Ca influx was much more sensitive to the calcium entry blockers as illustrated in Figure 5. As shown in Table 1, for both cinnarizine and flunarizine, IC_{50} and I_{50} values were of the same order of magnitude.

Discussion

$PGF_{2\alpha}$ and noradrenaline produced similar maximal

Table 2 Actions of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$ 33.3 μM) and noradrenaline (10 μM) on calcium influx and efflux in rat mesenteric artery measured as changes in ^{45}Ca resistant to displacement by lanthanum

	Tissue content of Ca^{2+} (μmol/kg wet wt.)		
		Influx of Ca^{2+}	
Control	73.8 ± 3.3 (6)	$PGF_{2\alpha}$	115.0 ± 4.2 (6)
Control	75.8 ± 3.5 (6)	Noradrenaline	107.3 ± 3.4 (6)
		Efflux of Ca^{2+}	
Control	162.8 ± 5.1 (8)	$PGF_{2\alpha}$	131.7 ± 8.2 (8)
Control	158.4 ± 9.4 (8)	Noradrenaline	98.3 ± 6.7 (8)

Values are given ± s.e.mean.

Control calcium influx was measured after 7 min incubation in ^{45}Ca containing physiological solution. Stimulated calcium uptake was measured by including either $PGF_{2\alpha}$ or noradrenaline in the incubating solution during the last 2 min. Control calcium efflux measurements were made after 2 h incubation in ^{45}Ca containing physiological solution followed by a 7 min wash in normal solution. Agonist effects on efflux were obtained by including either $PGF_{2\alpha}$ or noradrenaline in the last 2 min of the wash period. Numbers in parentheses represent number of arteries.

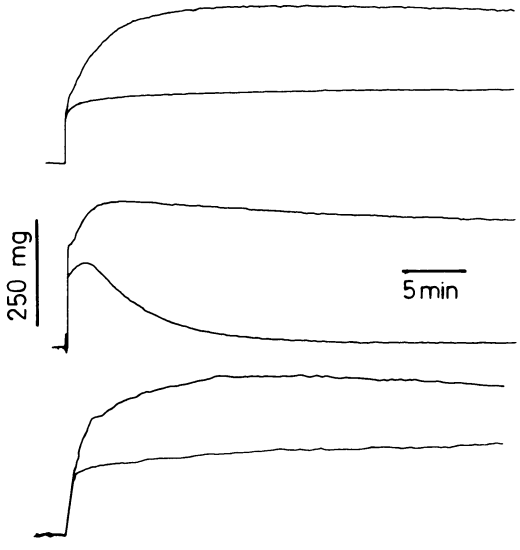

Figure 4 Characteristic depression of time-contraction responses obtained in rat mesenteric arteries produced by flunarizine. Contractions stimulated by noradrenaline 10 μM (top traces) and depolarization in physiological solution containing 100 mM KCl (middle traces) are shown before and after 90 min incubation with flunarizine 0.03 μM. The bottom traces depict contractions stimulated by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 33.3 μM before and after 90 min incubation with flunarizine 0.1 μM. Note the use-dependent inhibition of depolarization induced contractions by flunarizine. The calibration bars apply to all three pairs of responses.

contractions of the rat mesenteric artery in normal physiological solution. In calcium-free solution, a small contraction to noradrenaline was still evident but $PGF_{2\alpha}$ produced no response. A similar finding with $PGF_{2\alpha}$ has been reported in rat aorta (Altura & Altura, 1976), although in human arteries a $PGF_{2\alpha}$-mediated contraction still existed after 30 min exposure to a calcium-free medium (Mikhelson & Andersson, 1978). Others have shown that prostaglandins of the E type produce contractions of the rabbit aorta which are dependent on extracellular calcium (Levy, 1973; 1980; Wheeler & Weiss, 1980). Noradrenaline and adrenaline produce contractions of rabbit aorta and mesenteric artery, rat aorta and sheep carotid artery in calcium-free solution (Bohr, 1963; Godfraind & Kaba, 1969; Keatinge, 1972; Godfraind *et al.*, 1973; Karaki *et al.*, 1979; van Breemen & Siegel, 1980), although contractions of dog coronary and basilar arteries seem to be dependent on extracellular calcium (see van Breemen & Siegel, 1980).

Compared to $PGF_{2\alpha}$, noradrenaline produces a much faster initial rate of increase of tension and a significantly enhanced turnover of Ca^{2+} (i.e. influx plus efflux of Ca^{2+}) during the first 2 min of agonist contact (about 91.6 μmol for noradrenaline and 72.3 μmol for $PGF_{2\alpha}$). This higher turnover of Ca^{2+} evoked by noradrenaline is largely due to an enhanced rate of efflux and probably indicates a release of intracellulary stored calcium during this time. The faster rate of relaxation of noradrenaline compared to $PGF_{2\alpha}$-mediated contractions produced by EGTA also seems to be a reflection of these different early rates of Ca^{2+} efflux. However, the initially accelerated rates of Ca^{2+} turnover produced by these agonists are evidently not maintained, as intracellular ^{45}Ca accumulation measured after 60 min of agonist contact is the same for both $PGF_{2\alpha}$ and noradrenaline (unpublished observations).

Concentrations of cinnarizine and flunarizine producing 50% depression of $PGF_{2\alpha}$-mediated contrac-

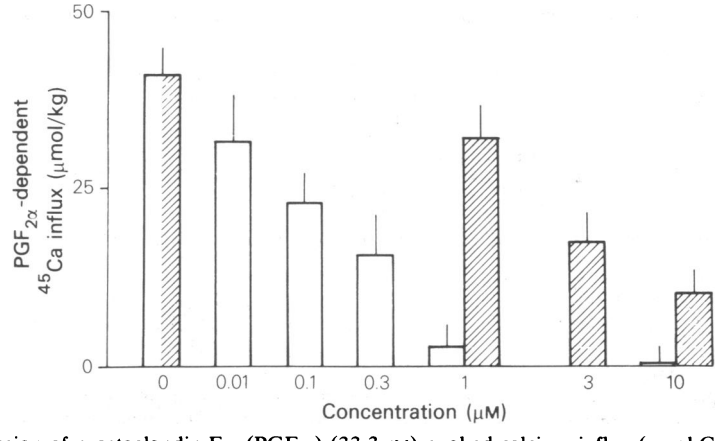

Figure 5 Depression of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (33.3 μM)-evoked calcium influx (μmol Ca/kg wet wt during 2 min) in rat mesenteric arteries by cinnarizine (1–10 μM) (hatched columns) and flunarizine (0.01–10 μM) (open columns). Each column is the mean of at least 6 observations, vertical lines represent s.e.mean.

tion and ^{45}Ca influx are similar (Table 1) and flunarizine 10 μM abolishes both effects, indicating that blockade of Ca^{2+} entry is probably responsible for the depression of contraction produced by these compounds. A similar correspondence between the depression of noradrenaline-stimulated contraction and calcium influx by flunarizine has been reported in the same tissue (Table 1) (Godfraind & Dieu, 1981), although noradrenaline-induced contractions are not completely inhibited by flunarizine, presumably due to a release of intracellular calcium.

It should also be noted that although flunarizine and cinnarizine inhibit the noradrenaline- and $PGF_{2\alpha}$-stimulated influx of ^{45}Ca in a concentration-dependent manner, they do not exert any similar effect on the resting influx of ^{45}Ca into control tissues during their 7 min incubation period, confirming previous observations (Godfraind, 1978; Godfraind & Dieu, 1981) which have been taken to indicate the presence of a separate Ca^{2+} leak channel.

Godfraind & Dieu (1981) also describe a use-dependent effect of flunarizine on contractions induced by depolarization which is characterized by an increase in the degree of inhibition of contraction with time of exposure to the depolarizing solution, a stable degree of inhibition begin achieved in about 20 min. This effect of flunarizine was not evident when noradrenaline was used as a contractile agent. No such use-dependent inhibitory effect of flunarizine was seen in experiments described here when mesenteric arteries were subjected to prolonged exposure to $PGF_{2\alpha}$ or noradrenaline, but was evident in depolarized tissues (Figure 4). This might mean that noradrenaline and $PGF_{2\alpha}$ do not depolarize the smooth muscle cell membrane of the rat mesenteric artery to a significant extent. Noradrenaline-mediated contraction in the absence of membrane depolarization has been observed in rabbit ear and saphenous arteries (Casteels, 1980; Holman & Surprenant, 1980) and Somlyo & Somlyo (1968) described contractions correlating poorly with membrane depolarization in vascular tissues from dog and rabbit.

Furthermore, there is an approximately 7 fold difference in the concentrations of flunarizine reported here to inhibit $PGF_{2\alpha}$-induced contraction and ^{45}Ca influx by 50% and those reported by Godfraind & Dieu (1981) to inhibit noradrenaline-induced effects. In the case of cinnarizine, an even greater difference is evident, it being about 50 fold more potent as an inhibitor of noradrenaline-than of $PGF_{2\alpha}$-induced contractions (Table 1). These differences could be interpreted by assuming the existence of two separate sets of Ca^{2+} entry channels, each activated by the interaction of the appropriate agonist with its particular receptor, the sets of channels having different affinities for flunarizine and cinnarizine. There are also possibly differences between the calcium channels activated by particular agonists in different tissues since $PGF_{2\alpha}$-induced contractions of the rat aorta are insensitive to both cinnarizine and flunarizine (unpublished observations). Alternatively, the receptor-operated Ca^{2+} channels could be identical, the difference being in the mode of coupling between receptor and calcium channel which would alter the apparent affinity of the channel for the calcium entry blocking agents.

In conclusion, this study shows that $PGF_{2\alpha}$ but not noradrenaline is entirely dependent on the entry of extracellular calcium to produce a contraction. Differing sensitivities to the calcium entry blockers, cinnarizine and flunarizine, suggest that $PGF_{2\alpha}$ and noradrenaline receptor-dependent calcium gating mechanisms might be different.

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