Br. J. Pharmacol. (1994), **113**, 1093–1098

Pharmacological reactivity of human epicardial coronary arteries: phasic and tonic responses to vasoconstrictor agents differentiated by nifedipine

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1 Human epicardial coronary artery rings, freshly obtained from cardiac transplantation patients, commonly exhibited phasic contractile activity *in vitro*. This activity occurred either spontaneously or in response to vasoconstrictor stimulation.

2 Nifedipine pretreatment (1 nM–0.1 μM) reduced both types of phasic contractions in a concentration-dependent manner. At 0.1 μM nifedipine, spontaneous contractions were completely abolished, as were phasic contractions induced by U46619, endothelin-1 or 5-hydroxytryptamine (5-HT).

3 For U46619 (0.1–100 nM), the largest phasic contractions (amplitude peak to trough) occurred over the mid-range of concentrations used (1–10 nM). At higher concentrations (30–100 nM), phasic activity was reduced as the response reached a maximum. Estimated pEC_{50} values for the upper phasic and lower phasic curves were significantly different (8.71 ± 0.13 versus 7.90 ± 0.11; $P<0.05$; $n = 10$). In the presence of nifedipine (0.1 μM), the purely tonic contraction curve to U46619 was similar to the lower phasic curve in the absence of nifedipine ($pEC_{50} = 8.14 \pm 0.06$, $n = 10$). Similar results were obtained for endothelin-1 (0.1–100 nM).

4 Responses to 5-HT (1 nM–3 μM) were more variable. The largest phasic contractions were spread unevenly throughout the concentration-response curve. In the presence of nifedipine (0.1 μM), the curve to 5-HT was significantly depressed in range but not sensitivity (pEC_{50}) when compared with the phasic curves.

5 In conclusion, activation of dihydropyridine-sensitive voltage-operated Ca^{2+} channels mediated the phasic contractions commonly observed in human epicardial coronary arteries. These contractions amplified the contractile responses to low concentrations of vasoconstrictors. Inhibition of phasic activity by the Ca^{2+} channel antagonist, nifedipine, allowed the tonic vasoconstrictor profile of human isolated coronary artery to be determined which is important information for the accurate quantitative assessment of vasodilator responses in this tissue *in vitro*.

Keywords: Human coronary artery; nifedipine; 5-HT; endothelin; thromboxane A_2; spontaneous phasic contractions; cromakalim

Introduction

Spontaneous phasic contractile activity in human isolated coronary artery has been reported by many researchers over the past fifteen years (Golenhofen, 1978; Ross *et al.*, 1980; Kalsner, 1985; Kimura *et al.*, 1989). Phasic activity is present in tissues of both cadaver and transplantation origin (Ginsburg *et al.*, 1980, 1984; Godfraind *et al.*, 1984; Verdernikov, 1986).

Whilst the mechanisms controlling tone in the human coronary artery are complex (see Ginsburg, 1984 for an overview), the phasic contractions are known to be dependent on Ca^{2+} entry (Ginsburg *et al.*, 1984). They are sensitive to various Ca^{2+} channel antagonists, including nifedipine, verapamil and diltiazem (Weinheimer *et al.*, 1983; Sjögren *et al.*, 1986; Kimura *et al.*, 1989) which abolish the phasic activity.

The occurrence of phasic activity, however, makes quantitative analysis of concentration-response curves to both vasoconstrictor and vasodilator agents in human isolated coronary arteries difficult (Verdernikov, 1986; Sjögren *et al.*, 1986). Therefore, the aim of this study was to use nifedipine to remove spontaneous phasic activity and to determine for the first time the tonic vasoconstrictor profile of human isolated coronary artery to the thromboxane A_2-mimetic U46619, endothelin-1 and 5-hydroxytryptamine (5-HT). A preliminary account of this study was presented at the 25th annual meeting of the Australian Society of Clinical and Experimental Pharmacologists and Toxicologists (Stork *et al.*, 1991).

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Methods

Human coronary arteries

Epicardial coronary arteries were obtained from the explanted hearts of 15 patients (12 male, 3 female) involved in the Alfred Hospital Heart & Lung Transplant Service who signed consent forms prior to surgery. Patients were diagnosed as having dilated cardiomyopathy ($n = 7$), ischaemic heart disease ($n = 5$), congenital heart disease ($n = 1$) or mitral valve disease ($n = 1$). One donor heart, unsuitable for transplantation, was also used. Average age was 45 years with a median age of 48 years (range 15–61).

Epicardial coronary arteries were dissected from hearts within 20 min of excision and taken to the laboratory in cold Krebs solution (4°C, see below for composition). Arteries were then further dissected free of any surrounding myocardium and fatty tissue and cut into 3 mm long ring segments. Only rings of arteries without gross macroscopic evidence of atherosclerosis were included. In total, 86 rings from 5 circumflex, 9 left anterior descending and 5 right coronary arteries, 2 primary circumflex and 3 primary left anterior descending coronary artery branches were used. Endothelium was left intact in all cases.

The vessel segments were set up in 30 ml organ baths containing Krebs solution [consisting of (in mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2 and glucose 11] at 37°C and aerated with a 95% O_2/5% CO_2 gas mixture. Two L-shaped wire hooks (355 μm in diameter) were passed through the vessel lumen. One wire was attached to a stationary support leg attached to a manually-driven micrometer, while the other wire was attached to an isometric force transducer (Grass Instruments, model FT03C) to measure force development. Force was recorded on either single (Rikadenki) or dual (W&W Scientific Instruments) flat bed pen recorders.

The arterial rings were allowed to equilibrate for 60 min before being subjected to two passive stretches to 5 g force at 30 min intervals (as per Chester *et al.*, 1990), which gives an resting tension optimal for contraction responses. Test drugs were then added and allowed to equilibrate for 30 min before cumulative concentration-response curves to the various vasoconstrictor agents were constructed at half-log unit intervals. Only one protocol and curve per ring per patient was performed.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate (5-HT), N^G-nitro-L-arginine (L-NOARG) (Sigma); U46619 [1,5,5-hydroxy-11α,9α (epoxymethano) prosta-E 2,13-dienoic acid] (Upjohn); endothelin-1 (Peninsula Laboratories, U.S.A.), (−)-nifedipine (Bayer) and cromakalim (Beecham).

Nifedipine (10 mM) was made up in 100% ethanol, and 5-HT (10 mM) in distilled water on the day of the experiment. L-NOARG-stock solution (0.1 M) was made up in 1 M $NaHCO_3$. All other drugs were from stock solutions made up in distilled water. All dilutions were in distilled water.

Statistics and data analysis

Contractile responses were measured and expressed in g force. In control vessels, where phasic activity occurred, both the top and bottom of the phasic contraction were measured separately for each agonist concentration (upper phasic and lower phasic, respectively). The individual concentration-response curves were computer-fitted to the sigmoidal logistic equation

$$Y = P_1 + P_2/[1 + e^{P_3(\log X - P_4)}],$$

where X = agonist concentration, P_1 = lower plateau response, P_2 = range between the lower and the maximal plateau of the concentration response curve, P_3 = a negative curvature index indicating the slope independently of the range and P_4 = log dose required to produce a half-maximal response (EC_{50}) (Elghozi & Head, 1990). These calculations were used to determine pEC_{50} values. All values are presented as the mean ± standard error of the mean (s.e.mean) for the given number of experiments (n).

One-way analysis of variance with Scheffe's test was used to make comparisons between the three individual groups (upper phasic, lower phasic and nifedipine-treated; Wallenstein *et al.*, 1980). The level of significance was set at $P \leqslant 0.05$.

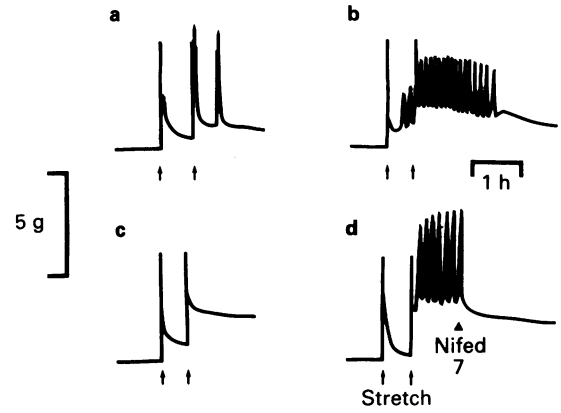

Figure 1 Traces of representative chart recordings of phasic contractile activity in human coronary artery; (a) irregular phasic contractions; (b) phasic activity with a regular rhythm; (c) quiescent arterial ring and (d) regular contractions abolished by nifedipine (Nifed, in − log M). Arrows indicate 5 g passive stretches.

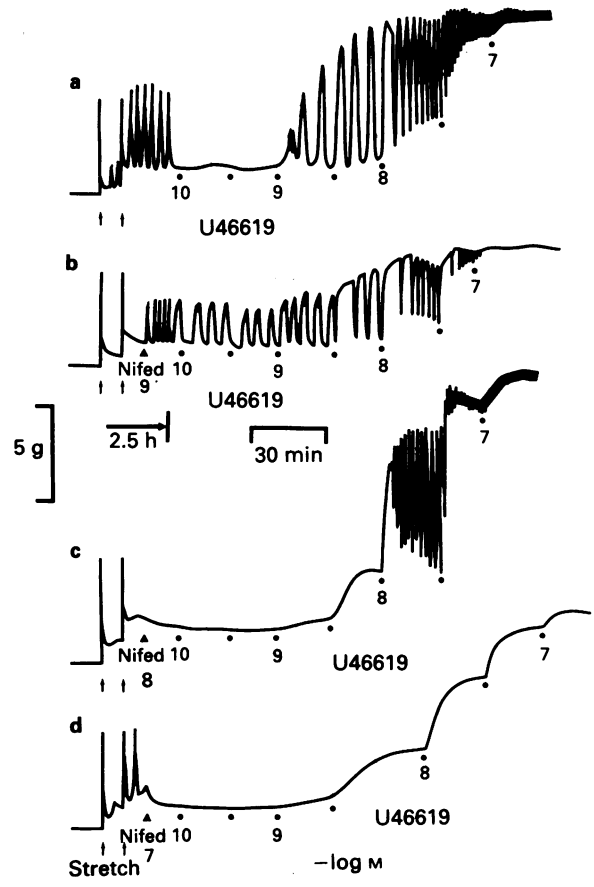

Figure 2 Effect of increasing concentrations of nifedipine on phasic activity and the concentration-response curve to U46619 in human coronary artery. Arterial rings were treated with concentrations (in − log M) of (a) nil; (b) 9; (c) 8 and (d) 7 nifedipine (Nifed). Traces are of chart recordings from 4 consecutive rings of a circumflex coronary artery from a 42 year old male. The recordings are representative of 4 separate experiments.

Results

Spontaneous phasic activity

Human isolated epicardial coronary artery ring segments commonly exhibited phasic contractile activity. These contractions occurred in nearly all ring segments, either spontaneously after the vessel was stretched or in response to vasoconstrictor stimulation (Figures 1, 2). The incidence, rhythm and amplitude of the spontaneous phasic contractions varied considerably, even between consecutive ring segments taken from the same artery. The amplitude of phasic contraction ranged between 1 and 7 g force and was observed to be independent of the level of passive force. Phasic activity was readily and rapidly removed by the addition of nifedipine (0.1 μM, Figures 1, 2).

Vasoconstrictor profiles

U46619 Tonic contractions to U46619 were masked by phasic contractile activity which was induced in quiescent arterial rings and enhanced in those which were already phasically active (Figure 2). Increasing concentrations of U46619 increased both the frequency (see Figure 2) and amplitude (see Figure 3) of the phasic contractions. At the higher concentrations (30–100 nM, U46619), the amplitude of the phasic contractions was reduced as the tonic contraction approached an apparent maximal response, but the frequency of phasic activity was increased to seconds per cycle (Figure 2). These U46619-induced phasic contractions were greatest in amplitide range (peak to trough) at 1–10 nM concentration (Figures 2a, 3b) and were blocked in a concentration-dependent manner by nifedipine (Figure 2).

In four separate experiments, consecutive arterial rings were exposed to either zero, 1, 10 or 100 nM nifedipine after which a cumulative concentration-response curve to the thromboxane A_2-mimetic U46619 was constructed (Figure 2). At zero and 1 nM nifedipine, phasic activity was seen in the U46619 curve on all occasions. In two experiments, 10 nM nifedipine completely abolished the phasic contractions, whilst in the other two experiments, the appearance of the phasic contractions was substantially delayed. In all four experiments, however, only tonic contractions were observed to U46619 stimulation in the presence of 0.1 μM nifedipine (Figure 2). Thus the amount of tonic contraction to U46619 was revealed in the presence of nifedipine (0.1 μM, Figure 3a). The tonic contraction curve for U46619 was superimposed on the curve for the lower level of the phasic contractions compared with the upper level curve, with all three curves (upper phasic, lower phasic and nifedipine-treated) converging at the highest concentration used (maximum response = 8.88 ± 1.16 g, 8.49 ± 1.28 g and 10.27 ± 1.54 g respectively, Figure 3a). Calculated pEC_{50} values for the lower phasic and nifedipine-treated curves were similar. The estimated value for the upper phasic curve, however, was significantly less ($P<0.05$, Table 1).

Sensitivity to U46619 did not differ between the main coronary vessels (circumflex and left anterior descending artery pEC_{50}s = 8.24 ± 0.10 and 8.15 ± 0.06; $n=3$ and 6, respectively) and their primary branches (pEC_{50} = 8.16 ± 0.17, $n=6$). Differences in maximum contractile force between the two groups of arteries were observed, but these could be attributed to differences in muscle mass (data not shown).

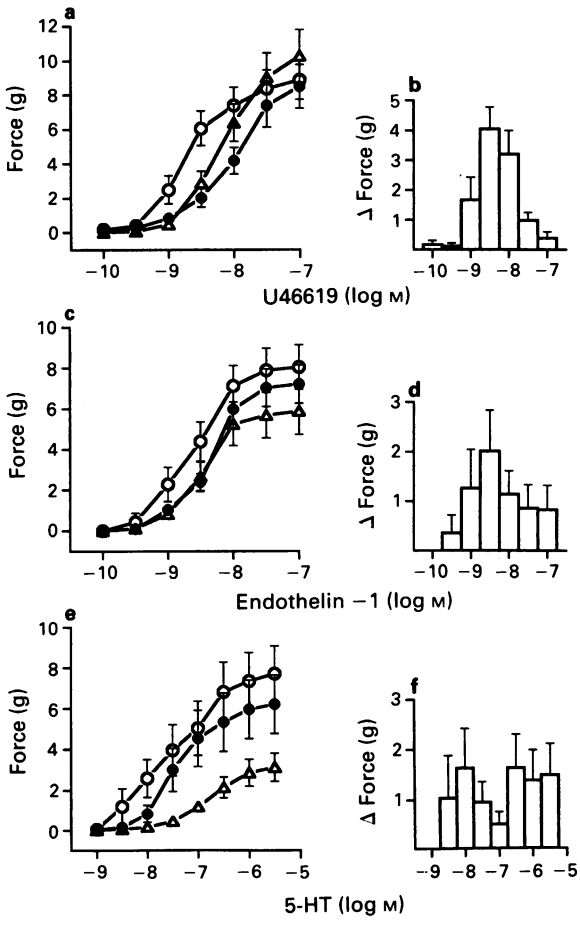

Figure 3 Response of human coronary artery to U46619, endothelin-1 and 5-hydroxytryptamine (5-HT). Mean cumulative concentration-response curves and the mean range of phasic contractions for each concentration were determined for (a) and (b) U46619; (c) and (d) endothelin-1 and (e) and (f) 5-HT in control [upper (○) and lower (●) levels of phasic contraction] and nifedipine-treated (0.1 μM, Δ) arterial rings. Mean pEC_{50} values for the curves are given in Table 1. Results are the mean ± s.e.mean of 10, 7 and 10 separate experiments for U46619, endothelin-1 and 5-HT, respectively

Endothelin-1 Results for endothelin-1 closely resembled those for U46619. Endothelin-1 induced phasic contractions in arterial rings not treated with nifedipine, which again made determination of the tonic contraction curve difficult (Figure 3c). Again, the amplitude of the phasic activity was greatest in the mid-range of concentrations; 1–10 nM (Figure 3d). Nifedipine (0.1 μM)-treated tissues contracted only tonically to endothelin-1. The concentration-tonic contraction curve in the presence of nifedipine closely followed the lower phasic curve in the phasically active tissues. There was a small but significant reduction in the maximal response between the upper phasic and nifedipine-treated curves (7.90 ± 1.08 g vs. 5.70 ± 1.12 g, $P<0.05$). There was also a significant difference between the pEC_{50} estimates for the upper phasic and lower phasic curves ($P<0.05$, Table 1).

5-HT Responses to 5-HT were the most variable. In the absence of nifedipine, phasic contractions reached a peak amplitude of 7.69 ± 1.08 g at 3 μM 5-HT (Figure 3e). The maximum contraction in the presence of nifedipine (0.1 μM) was significantly less (3.10 ± 0.69 g, $P<0.05$). The range in amplitude of the phasic contractions did not follow the pattern seen with U46619 and endothelin-1 where the greatest responses occurred in the mid-range concentrations. Rather, they were spread throughout the 5-HT concentration-response curve (Figure 3f). There was no significant difference between the pEC_{50} values for the three groups (upper phasic, lower phasic and nifedipine-treated) ($P<0.05$, Table 1).

Other drugs The ability of the ATP-sensitive K^+ channel opener, cromakalim and the L-arginine analogue N^G-nitro-L-arginine (L-NOARG) to abolish the phasic contractile activity was also examined.

Cromakalim (0.3 μM, in preliminary studies found to be a submaximal concentration for relaxation) initially removed any spontaneous phasic activity from the arterial ring segments but failed to stop induction of phasic contractions by vasoconstrictor stimulation with U46619 ($n=4$).

Table 1 Calculated pEC_{50} values for three vasoconstrictor drugs in human coronary artery

	Upper phasic	*Lower phasic*	*Nifedipine*
U46619	8.71 ± 0.13* (9)	7.90 ± 0.11 (10)	8.14 ± 0.06 (10)
Endothelin-1	8.75 ± 0.16 (7)†	8.34 ± 0.14 (7)†	8.45 ± 0.10 (7)
5-HT	7.31 ± 0.27 (8)	7.16 ± 0.18 (9)	6.80 ± 0.11 (9)

Values are mean ± s.e.mean for (n) experiments. *Indicates values significantly different from all others; †indicates values significantly different from each other ($P<0.05$, ANOVA).

Cromakalim pretreatment did not significantly affect the U46619 concentration-response curve (data not shown).

L-NOARG (0.1 mM) had no effect on either phasic contractile activity or U46619-induced tone ($n = 4$, data not shown).

Discussion

Human isolated epicardial coronary arteries commonly exhibited phasic contractile activity either spontaneously upon stretch or with vasoconstrictor stimulation. These contractions were abolished by nifedipine in a concentration-dependent manner but were not removed by cromakalim or the L-arginine analogue N^G-nitro-L-arginine (L-NOARG). Thus nifedipine (0.1 μM) allowed the determination of the vasoconstrictor profile of the thromboxane A_2-mimetic U46619, endothelin-1 and 5-HT in this tissue.

It could be argued that the phasic contractions observed in this and previous studies (see Introduction for references) are indicative of diseased hearts. However, in this study and others we have performed (Cocks *et al.*, 1993) vessels obtained from unused donor hearts have also been observed to display phasic contractile activity. The observation of a wide variation in phasic contractions amongst arterial rings, even within the same artery, has been previously reported by both Ross *et al.* (1980) and Verdernikov (1986). The reason for the variability in phasic activity, however, remains unknown.

Nifedipine, a dihydropyridine, voltage-operated Ca^{2+} channel antagonist, which acts by preventing the opening of the ion channel in response to depolarization of the smooth muscle membrane, removed phasic activity and unmasked stable, tonic contractions to vasoconstrictor stimulation. Golenhofen (1978) and Weinheimer *et al.* (1983) have previously reported that spontaneous activity in human isolated coronary artery was reduced and rhythmic fluctuations abolished by nifedipine in the range of 1 nM–1 μM. Similar results have been reported for two other Ca^{2+} channel antagonists, verapamil (Ross *et al.*, 1980; Verdernikov, 1986; Sjögren *et al.*, 1986) and diltiazem (Ginsburg *et al.*, 1980; Kimura *et al.*, 1989). All three Ca^{2+} antagonists are also effective against rhythmic contractions in monkey isolated coronary arteries (Ishii *et al.*, 1985), in concentrations similar to those required in human tissue.

Sjögren *et al.* (1986) noted that '. . . rhythmic activity in the human coronary arteries *in vitro* to some extent renders pharmacological experiments impossible'. In the present study we were able to determine the vasoconstrictor profile of U46619, endothelim-1 and 5-HT in the presence and absence of nifedipine (0.1 μM). In the absence of nifedipine, the phasic contractions appeared to be added on top of the tonic contractions for all three agonists. This was previously noticed by Godfraind *et al.* (1984) for 5-HT, but seemingly not followed up. Addition of the phasic contractions on the tonic contractions was confirmed by the fact that in the presence of nifedipine, the tonic contraction curves to U46619 and endothelin-1, at least, were superimposed on the curves for the lower level of phasic activity.

The results for U46619 closely matched those for the stable analogue carbocyclic thromboxane A_2 in similar preparations (Toda, 1984). The reason for the difference in pEC_{50} values for U46619 between the upper phasic curves and the lower phasic and nifedipine-treated curves is unclear but may indicate that the phasic contractions are more sensitive than tonic contractions to this vasoconstrictor stimulus.

Endothelin-1 also caused contraction of human coronary artery, possibly via receptors located predominantly on the adventitial side of the tunica media (Chester *et al.*, 1989), with a maximal contraction of 0.1 μM. The maximal contraction to endothelin-1 was 6.56 ± 0.77 g (64.35 ± 7.55 mN, n = 14, combining our results for the lower phasic and nifedipine-treated curves), approximately double that of Chester *et al.* (1989; 38.7 ± 6.4 mN, $n = 34$). Estimates for pEC_{50} values for endothelin-1 also differ between the two studies. Whilst not documented, the data of Chester *et al.* (1989) indicates a pEC_{50} value of approximately 7.7, about an order of magnitude lower than that found here (8.45 in the presence of nifedipine, 0.1 μM). Our pEC_{50} value for endothelin-1 in the human coronary artery is similar to pEC_{50} estimates in human internal mammary artery (8.60, Lüscher *et al.*, 1990; 8.01, Costello *et al.*, 1990), human saphaneous vein (8.40, Lüscher *et al.*, 1990; 8.12, Costello *et al.*, 1990) and porcine coronary artery (approximately 9.3, Yanagisawa *et al.*, 1988). As with U46619, the difference in pEC_{50} values for endothelin-1 between the phasic curves probably reflects the relative sensitivity of phasic activity to vasoconstrictors.

The reduction of the maximal contraction to endothelin-1 by nifedipine (and nicardipine, see Chester *et al.*, 1989) may be due to a decrease in Ca^{2+} entry via voltage-operated Ca^{2+} channels, since Yanagisawa *et al.* (1988) showed the responses to endothelin-1 are sensitive to Ca^{2+} channel antagonists. Endothelin-1, however, also activates the phosphatidylinositol second messenger system which has also been implicated in the contraction responses to this peptide (Pang *et al.*, 1989; Kasuya *et al.*, 1989).

5-HT stimulates both 5-HT_1-like and 5-HT_2 receptors to contract the human coronary artery and is the most efficacious of a range of 5-HT receptor antagonists in this tissue (Connor *et al.*, 1989; Chester *et al.*, 1990; Angus, 1990; Cocks *et al.*, 1993; Bax *et al.*, 1993). Both receptor subtypes appear to be important in mediating the hyperreactivity to 5-HT associated with coronary artery disease (Chester *et al.*, 1990; Cocks *et al.*, 1993; McFadden *et al.*, 1991). The 5-HT responses in the presence of nifedipine (3.10 ± 0.69 g at 3 μM) were generally equal to, or greater in contractile strength than those seen in previous studies (Chester *et al.*, 1990; Kalsner & Richards, 1984; Kalsner, 1985).

In the absence of nifedipine, the maximum force developed was significantly greater than the maximum contractile response in the presence of nifedipine. This may indicate that the response to 5-HT was not only augmented by the phasic activity, but also that part of the tonic contraction of 5-HT was blocked by nifedipine. Angus & Brazenor (1983) have shown that nifedipine is a more selective relaxant of submaximal 5-HT- then U46619-induced tone in dog large coronary artery, while in rabbit basilar artery, the contractile but not the depolarization response to 5-HT is sensitive to voltage-operated Ca^{2+} antagonists (Clark & Garland, 1993).

Phasic contractions of a similar magnitude developed to 5-HT over the whole range of the concentration-response curve. This contrasts with the pattern of the phasic contractions seen to U46619 and endothelin-1, where there was a bell-shaped distribution of the amplitude of phasic contractions and the phasic activity was added on top of the tonic contractions. Such amplification of the contractile responses to vasoconstrictor agonists by phasic activity may be important clinically in cases of arterial spasm. Maseri *et al.* (1990) suggested that segmental arterial spasm is caused by local hyperresponsiveness to a range of vasoconstrictors, including ergonovine (a 5-HT receptor agonist). Functional synergy may also be involved as we have previously demonstrated that in human epicardial coronary arteries *in vitro*, low concentrations of U46619 increase the contractile response to 5-HT and other 5-HT agonists (sumatriptan, ergometrine and methysergide) but do not affect the sensitivity to these agonists (Cocks *et al.*, 1993, see also Chester *et al.*, 1993). The synergistic interaction also occurs with noradrenaline (Cocks and Kemp, unpublished data) and for both noradrenaline and 5-HT with threshold concentrations of endothelin-1 (Yang *et al.*, 1990).

Our results in this study indicate that phasic activity can also augment the contractile response to a range of vasoconstrictors, particularly at low and medium concentrations. The mechanism behind this synergy remains to be elucidated,

although it would appear to be at a second messenger level, rather than the receptor level, since a range of agonists, and therefore receptor mechanisms, are implicated. In the case of amplification by phase activity, it is likely that the changing intracellular Ca^{2+} levels 'prime' the contractile processes to respond, hence increasing responsiveness, particularly to low vasoconstrictor concentrations. Clinically, our results implicate phasic activity in arterial spasm and indicate the use of nifedipine or other voltage-operated Ca^{2+} channel antagonists in both prophylactic and acute treatment.

In summary, phasic contractile activity was commonly seen in human coronary arterial rings *in vitro*. This activity involved activation of voltage-operated Ca^{2+} channels. Nifedipine removed the phasic contractions in a concentration-dependent manner and allowed determination of tonic vasoconstrictor profiles in the human coronary arteries for the thromboxane A_2-mimetic U46619, endothelin-1 and 5-HT. These results suggest that, under appropriate *in vivo* conditions, phasic activity could amplify contractile responses to low concentrations of vasoconstrictor drugs, such as 5-HT and thromboxane A_2, which are implicated in coronary artery spasm (see Kalsner, 1982; Maseri *et al.*, 1990; McFadden *et al.*, 1991; Cocks *et al.*, 1993; Chester *et al.*, 1993). This study has also established conditions under which vasodilator responses can be studied optimally (see Stork & Cocks, 1994).

We gratefully acknowledge the assistance of Mr Don Esmore, Mr Robin McEgan, Mr Arthur Preovolos and all the team of the Alfred Hospital Heart & Lung Transplant Service. Cromakalim was a gift from Beecham and Dr Grant McPherson. This work was supported by institute grants from the National Health and Medical Research Council of Australia and Glaxo Australia.

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(Received September 20, 1993
Revised February 16, 1994
Accepted February 17, 1994)