5-HT_{1D} receptor agonists and human coronary artery reactivity *in vitro*: crossover comparisons of 5-HT and sumatriptan with rizatriptan and L-741,519

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- 1 Rizatriptan (MK-462, (*N,N*-dimethyl-2-[5-(1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethylamine)) and its structurally related analogue L-741,519 (*N*-methyl-4-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]piperidine) are novel 5-HT_{1D}-receptor agonists. Rizatriptan has shown efficacy as an anti-migraine agent in clinical trials. Since angiographic studies in patients have shown that sumatriptan (an established 5-HT_{1D}-receptor agonist) can cause coronary artery vasoconstriction, we compared the effects of rizatriptan and L-741,519 with those of 5-HT and sumatriptan on endothelium-denuded segments of human coronary artery *in vitro*.
- 2 Coronary arteries were obtained from explanted hearts from patients undergoing cardiac transplantation ($n=16$ viable arteries from 13 males, 3 females, aged 38–68 years) and arterial segments (5–6 mm in length) were mounted in organ baths for isometric tension recording. Each segment was first exposed to 45mM KCl and then to 5-HT (1 nM–100 μ M). Concentration–effect curves to rizatriptan and sumatriptan (Study 1, $n=6$ or 7 arteries) or sumatriptan and L-741,519 (Study 2, $n=8$ arteries) were then performed in a consecutive and random manner. The response to repeated application of 5-HT was obtained in separate segments.
- 3 One artery showed severe atheroma and was not included in the analysis. ANOVA showed that 5-HT responsiveness varied significantly between arteries from different patients, but not between arterial segments from the same patient. Desensitization was seen consistently across all agonists but did not significantly affect inter-agonist comparisons.
- 4 There was graded effectiveness in the ability of the agonists to cause contraction with the rank order of $E_{\max}$ values being 5-HT $\gg$ sumatriptan $>$ L-741,519 $>$ rizatriptan. In terms of EC_{50} values, L-741,519 was significantly more potent than sumatriptan.
- 5 The present study (using a ‘cross-over’ experimental protocol) confirms our previous observation that rizatriptan is less effective than sumatriptan in causing contraction of human isolated coronary artery. Furthermore, it shows that the lower maximum contractile response to rizatriptan, compared with that of sumatriptan, is not merely the consequence of variability in response to 5-HT_{1D}-receptor agonists between patients or between segments from the same artery.

Keywords human coronary artery coronary vasoconstriction 5-HT_{1D}-receptor agonists

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Introduction

It is well established that human isolated coronary artery can both contract and relax in response to 5-hydroxytryptamine (5-HT) [1, 2]. Thus 5-HT can give rise to either coronary vasoconstriction or coronary vasodilation depending on the balance between these two opposing effects. In angiographic studies, 5-HT increased coronary artery diameter and blood flow in normal subjects but in patients with coronary artery disease, this response was lost and a vasoconstrictor response was seen [3, 4]. These effects of 5-HT can be mediated via 5-HT_{1D}- or 5-HT₂-receptor activation [1, 2, 5, 6].

In angiographic studies, intravenous administration of sumatriptan (a selective 5-HT_{1D}-receptor agonist) to patients caused a decrease in coronary artery diameter. However, this effect of sumatriptan was not accompanied by any clinical symptoms or changes in ECG [7]. Some clinical trials involving the assessment of sumatriptan as an anti-migraine agent have reported that 'chest-tightening' may be experienced by some subjects and coronary ischaemic episodes (including myocardial infarction and at least one death) have been reported [8, 9, 10, 11]. However, it should be noted that this type of serious event is rare. Recently, it has been suggested that the relatively common 'chest-tightening' symptoms may be related to oesophageal discomfort rather than to effects on the heart [12].

Rizatriptan (MK-462, (*N,N*-dimethyl-2-[5-(1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethylamine)) and its chemically related analogue L-741,519 (*N*-methyl-4-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]piperidine) are novel 5-HT_{1D}-receptor agonists [13, 14, 15]. Rizatriptan has been effective in clinical trials to date as an antimigraine agent [16]. In a previous study [17], the effect of rizatriptan on human isolated coronary arteries was compared to those of sumatriptan and 5-HT; rizatriptan produced a smaller maximum contraction than did 5-HT or sumatriptan. In this study [17], one segment from each artery studied was exposed to a single agonist (either 5-HT, sumatriptan or rizatriptan). Recently, it has been reported that the contribution of 5-HT_{1D}-receptor activation to the contraction of human coronary artery shows considerable variation between segments taken from the same piece of artery, and can depend on the presence or absence of atheroma [5, 18]. It is therefore possible, that in the study by Ferro *et al.* [17], differences in the responses to rizatriptan and sumatriptan may be explained, in part, by differences in the responsiveness of individual artery segments.

The primary aim of the present study was to compare the effects of rizatriptan on human isolated coronary artery with those of 5-HT and sumatriptan using a cross-over experimental design to enable direct comparison of contractile effects on the same arterial segment, thus eliminating any regional variation of 5-HT_{1D}-receptor responsiveness. In addition to using a different experimental design to that employed by Ferro *et al.* [17], the present experiments also used a different patient population for the source of coronary arteries

since heart transplant recipients were patients in the USA. Comparisons of the effects of L-741,519 with the effect of sumatriptan and 5-HT were also made.

The study was approved by the Ethics Committee, Baylor Medical College, Texas and a preliminary communication of part of the study has been presented to the British Pharmacological Society [19].

Methods

Patients

Coronary arteries (left descending, left circumflex or right coronary arteries) were obtained from 19 patients undergoing orthotopic cardiac transplant. Usable data were obtained from coronary artery rings of only 16 of these 19 patients; rings from the other three patients were not viable and these patients are not included in the demographic summary. The average age (range) of these 16 patients was 55 years (38–68 years). Ten patients had ischaemic cardiomyopathy and the remaining six had dilated cardiomyopathy. There were 13 males and three females in this group; two patients were African-American and 14 were Caucasian. Patients received a variety of medications prior to transplant. A majority (9/16) were receiving cardiac inotropic support with either dobutamine or dopamine or both. Most patients were also being treated with angiotensin converting enzyme inhibitors (9/16) and many were also on diuretics (11/16) and nitrates (8/16). Some patients were receiving anti-arrhythmics (amiodarone, milrinone, vesnarinone). One patient was taking a serotonin reuptake inhibitor (fluoxetine). Except for one patient who received OKT3 (a monoclonal antibody for immunosuppression) at the time of transplantation, all other patients were pre-treated just prior to transplantation with cyclosporine and azathioprine. Other medications taken by a minority of patients included antibiotics, β_2 -adrenoceptor agonists, antiviral agents, histamine-receptor antagonists, oral hypoglycaemics, anxiolytics and hypnotics, glucocorticoids, cholesterol lowering agents, anti-coagulants, lactulose, mannitol, aspirin, potassium chloride and magnesium oxide.

Preparation of arterial segments

Each artery was dissected from the heart immediately following explantation and was then kept at 4°C (0.5–7 h) in modified Krebs's physiological salt solution (composition in mM: NaCl 118.3, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, glucose 11.1, CaCl₂ 2.5; control solution) prior to commencing the experiment. The arteries were cleaned of connective tissue and the endothelium removed mechanically with the tip of watchmaker's forceps. Each artery was cut into ring segments (each approximately 5–6 mm in length) with between three to eight segments being obtained from each artery. Each segment was mounted for isometric

tension recording in tissue baths containing control solution aerated with 95% O₂ and 5% CO₂, maintained at pH 7.4 and temperature 37°C. The segments were stretched in a stepwise manner until they reached the maximum of the length-tension curve, which was determined by a comparable response to 45 mM KCl at two different levels of stretch. The segments were then allowed to equilibrate to their own basal tension level.

Experimental procedure

For all segments from the same artery, contractions to 45 mM KCl were first elicited and this response was used as a reference. To test for 5-HT responsiveness a cumulative concentration–effect curve to 5-HT was then obtained in all segments (Curve 1). The segments were then randomly allocated to one of three groups (see Figure 1) and subjected to two further consecutive agonist concentration–effect curves: in Study 1; for

segments assigned to Group 1, 5-HT was used as the agonist (Curves 2 and 3); for segments assigned to Group 2 the order of application of the 5-HT_{1D}-receptor agonists was sumatriptan (Curve 2) followed by rizatriptan (Curve 3) and for segments assigned to Group 3, the order of agonist application was MK-462 (Curve 2) followed by sumatriptan (Curve 3). A similar cross-over design with respect to sumatriptan and L-741,519 was used in Study 2 (see Figure 1). For each agonist, the response to each concentration was allowed to plateau (5–6 min) before addition of the next concentration. If no response was observed after 3 min, the next concentration of the agonist was added to the organ bath. Further additions of agonists were suspended on attaining the maximum contractile response (i.e. where addition of a higher agonist concentration no longer produced a further increase in tension) or in cases, where no clear maximum response could be defined, the maximum concentration of agonist added was 100 µM. Following each concentration–effect curve the segments were washed several

Figure 1 Schematic representation of the experimental protocol and the order of agonist application for Study 1 (depicted in the upper section) and Study 2 (depicted in the lower section). Multiple segments were obtained from each coronary artery and randomly assigned to one of three groups. Each segment was then subjected to three agonist concentration–effect curves, with the agonists used being determined by Group-assignment: for both studies, all segments were subjected to an initial concentration–effect curve to 5-HT and then segments assigned to Group 1 were subjected to two further consecutive 5-HT concentration–effect curves; segments assigned to Groups 2 and 3 were also subjected to two further concentration–effect curves, with the order of application of sumatriptan and rizatriptan (Study 1) or sumatriptan and L-741,519 (Study 2) being reversed for Groups 2 and 3. The boxes indicate data sets which were pooled to obtain mean responses to each of the 5-HT receptor agonists. For example, in both Study 1 and Study 2 the mean sumatriptan response was obtained by pooling data in the second and third columns.

times in control solution until baseline tone was re-established.

Analysis of data

Comparison of overall effects of the agonists using analysis of variance (ANOVA) Responses to 5-HT, sumatriptan and rizatriptan or L-741,519 were measured in g tension and expressed as a percentage of the response to 45 mM KCl. In some segments, contractile responses to the 5-HT_{1D}-receptor agonists were 'phasic' in nature (see Figure 2) and these contractions were measured in a similar way to 'tonic-type' contractions, with the response being measured as the maximum tension achieved in the presence of each agonist concentration. The data were subjected to a series of ANOVAs: (a) analysis of data from the first concentration-effect curve to 5-HT (to test for responsiveness to 5-HT and to examine inter-individual variability between individual segments obtained from the same coronary artery and between coronary arteries obtained from individual patients); (b) analysis of agonist evoked responses obtained for Curves 2 and 3 (to test for desensitization); (c) analysis of data obtained on second and third concentration-effect curves for comparison of the effects of 5-HT, sumatriptan, rizatriptan and L-741,519. These analyses were performed using BMDP statistical software (University of California Press, Los Angeles, USA) specialized for mixed and repeated measures designs, and which provide a method for testing nonlinear effects on repeated measures (agonist concentration). For the purpose of these ANOVAs a number of different dependent variables were used to describe aspects of the concentration-related response, including means, maximums, areas under the concentration-effect curve and a linearisation of the slope of this curve. Results were consistent no matter which measure was used and we report those for area under the curve and mean as appropriate.

Comparison of concentration-effect curve parameters ($E_{\max}$ and EC_{50} values) using non-linear regression analysis All inter-agonist comparisons were made by pooling data obtained in Curves 2 and 3 (see Figure 1). Therefore for each artery (since multiple segments from each artery were used), mean responses to 5-HT were calculated by pooling data obtained in Curves 2 and 3 for segments assigned to Group 1. In addition (due to the cross-over design of the order of agonist application), mean responses to sumatriptan, rizatriptan or L-741,519 were calculated by pooling data obtained in Curves 2 and 3 across segments assigned to Groups 2 and 3 (see Figure 1). Then concentration-effect curves were fitted to the data using least squares non-linear regression analysis and the equation $E = E_{\max} / (1 + (EC_{50}/\text{agonist concentration})^{nH})$, where $E_{\max}$ is the maximal contraction evoked by each agonist (relative to 45 mM KCl), EC_{50} is the half maximally effective concentration and nH is the Hill coefficient, using Grafit Version 3.0 (Erithacus Software Ltd, Staines, UK). In addition, for all arteries tested in Study 1 and all arteries tested in Study 2, the

data were pooled and the overall mean data for each agonist were calculated. Concentration-effect curves were then fitted to this overall mean data, using weighted least squares non-linear regression analysis and the same equation using BMDP statistical software (University of California Press, Los Angeles, USA). The weighting was the reciprocal of the error estimate for each mean value. Differences between the estimates $E_{\max}$ and EC_{50} values obtained for the agonists (i.e. derived from curve fitting to the overall mean data sets) were tested using asymptotic *t*-tests and error estimates from the fitted results.

Materials

5-HT was obtained from Sigma (St Louis, MO, USA). Rizatriptan (benzoate salt) and L-741,519 (benzoate salt, *N*-methyl-4-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]-piperidine) and sumatriptan (succinate salt) were synthesized by Merck Sharp & Dohme Research Laboratories, Harlow, UK.

Results

Coronary arteries from 19 patients were studied, but arteries from three patients did not respond to 45 mM KCl and were considered non-viable. Arteries from Patients 1 and 2 showed visible signs of minor atherosclerosis whilst the artery from Patient 3 showed major atherosclerosis and data from this vessel were excluded from the statistical analyses. For most arterial segments, 5-HT and the 5-HT_{1D}-receptor agonists produced tonic (maintained) contractions. Whereas in Study 1 some arterial segments showed a phasic contraction (see Figure 2 for typical examples of tonic and phasic contractions to 5-HT), phasic contractions were rarely seen in Study 2. For Study 1, statistical analysis (area under the concentration-effect curve) showed that contractions seen in segments which displayed 'tonic' activity were significantly greater in magnitude than contractions seen in segments which displayed 'phasic' activity ($P < 0.004$). A coded variable was used to represent this effect and to remove it from the dependent variables analysed. This analysis of covariance established that the nature of the contractile response (i.e. tonic or phasic) did not effect the outcome of the comparisons between the effects of the 5-HT receptor agonists.

It was anticipated that patient medication at the time of heart transplantation would have little or no effect on agonist evoked contractile responses, since few of the medications interact directly with 5-HT-receptors. In addition, the arterial segments were washed several times in physiological buffer which is likely to remove any residual drugs. A preliminary statistical analysis was performed and no significant effects were seen although since each patient received a different cocktail of drugs the database was limited.

Figure 2 Isometric tension recordings of contractile concentration-effect curves to 5-HT in two separate endothelium denuded coronary artery segments (from Patient 5). Additions of 5-HT are indicated by the vertical bars. The upper panel shows a 'tonic' or maintained contractile response to 5-HT, and the lower panel shows a 'phasic' type contractile response to 5-HT. In each panel the horizontal dotted line indicates the basal tension and the vertical dotted arrows show examples of the measurement of changes in tension (either 'tonic' and 'phasic') evoked by agonists.

Comparison of agonist responses using analysis of variance

Analysis of responses to 5-HT (Curve 1) For all arteries, initial exposure to 5-HT caused a significant contractile response (Study 1: $F=124.7$, d.f.=1,38; $P<0.0001$; Study 2: $F=119.61$, d.f.=1,42; $P<0.0001$). There was no significant difference in 5-HT responsiveness between segments from the same artery (Study 1: $F=0.942$, d.f.=7,38; $P=0.47$; Study 2: $F=0.401$, d.f.=7,42; $P=0.40$). However, there was a highly significant difference in the level of response to 5-HT between arteries obtained from different patients (Study 1: $F=9.60$, d.f.=6,101; $P<0.0001$; Study 2: $F=3.04$, d.f.=7,42; $P=0.011$). Furthermore, the variability in 5-HT responsiveness between segments from the same artery differed significantly from patient to patient (Study 1: $F=2.20$, d.f.=7,11; $P=0.04$; Study 2: $F=2.20$, d.f.=7,131; $P=0.039$). The statistical tests were made using three dependent variables (mean, (the reported F values), maximum observed response and area under the concentration-effect curve) and produced essentially the same results.

Analysis of possible desensitization to repeated exposure to the agonists (Curves 2 and 3) Analysis of variance (means, using pooled data from Studies 1 and 2) showed

there was significant desensitization of agonist-evoked responses obtained in Curves 2 and 3 ($F=45.59$, d.f.=3,128; $P<0.0001$). However, this desensitization applied consistently across all agonists, and the interaction between agonist and Curve number (i.e. 2 or 3) was not significant (i.e. agonist $\times$ Curves 2 or 3 interaction, $F=1.95$, d.f.=3,128; $P=0.1248$), indicating that desensitization did not influence comparison of agonist effects.

Comparison of responses to 5-HT, sumatriptan, MK-462 and L-741,519 (Curves 2 and 3) Post analysis of variance t -tests showed that for Study 1 the responses to both rizatriptan and sumatriptan were significantly lower than that for 5-HT (means: $t=3.44$, d.f.=34; $P=0.002$ and $t=4.33$, d.f.=34; $P=0.001$ respectively) and similarly for Study 2, where responses to L-741,519 and sumatriptan were significantly lower than those to 5-HT (means: $t=6.64$, d.f.=76; $P<0.0001$; $t=6.64$, d.f.=76; $P<0.0001$ respectively). There were no other significant differences between agonists. When statistical power was increased by pooling the data for both studies, the results for the contrasts for all compounds with 5-HT were essentially the same, except that in addition the difference between the effects of rizatriptan and sumatriptan was revealed as significant (means: $t=2.87$, $P=0.005$; area under the concentration-effect curve $t=2.95$, $P=0.004$; observed maximum of curve $t=2.180$, $P=0.024$); however, L-741,519 did not differ from sumatriptan.

Comparison of concentration-effect curves parameters (E_{max} and EC_{50} values) using non-linear regression analysis Multiple segments from each artery were exposed to the agonists. The mean responses to 5-HT, sumatriptan, rizatriptan or L-741,519 for all segments from each artery are shown in Figures 3 and 4 and the estimated E_{max} and EC_{50} values derived from curve fitting to this data are shown in Table 1. In addition, the overall mean data for each agonist obtained for all arteries tested in Study 1 and Study 2 are shown in Figure 5a and 5b respectively and the estimated E_{max} and EC_{50} values derived from curve fitting to the overall mean data are shown in Table 1.

Statistical comparison of data obtained from Study 1 (i.e. direct comparison of rizatriptan and sumatriptan on the same arterial segment) showed that the overall E_{max} values for both sumatriptan and rizatriptan were significantly lower than that for 5-HT and furthermore the overall E_{max} value for rizatriptan was significantly less than that for sumatriptan ($t=8.59$, d.f.=15; $P<0.001$) whereas the overall EC_{50} values for all three agonists were not significantly different. For Study 2 (i.e. direct comparison of L-741,519 and sumatriptan), the overall E_{max} values for L-741,519 and sumatriptan differed significantly from that obtained for 5-HT and the overall E_{max} value for L-741,519 was significantly lower than that for sumatriptan ($t=10.915$, d.f.=12; $P<0.001$). Furthermore the overall EC_{50} value for L-741,519 was significantly less than that for sumatriptan ($t=13.43$, d.f.=12; $P<0.001$), indicating that L-741,519 was more potent than sumatriptan.

There was a significant difference between the E_{max}

Figure 3 Concentration-effect curves to 5-HT (closed squares), sumatriptan (closed circles) and rizatriptan (open squares) for each artery. Points represent mean data (obtained from Curves 2 and 3) from all arterial segments ($n=2-5$ segments from the same artery) and vertical bars signify $\pm$ s.e. mean (where $n>2$). Curves were fitted by weighted least squares non-linear regression analysis.

Figure 4 Concentration-effect curves to 5-HT (closed squares), sumatriptan (closed circles) and L-741,519 (open triangles) for each artery. Points represent mean data (obtained from Curves 2 and 3) from all arterial segments ($n=2-5$ segments from the same artery) and vertical bars signify $\pm$ s.e. mean (where $n>2$). Curves were fitted to the mean data obtained from each artery by weighted least squares non-linear regression analysis.

Table 1 Estimated values of $E_{\max}$ (% 45 mM KCl) and EC_{50} (μM) calculated using non-linear regression analysis. For individual patients, estimated $E_{\max}$ and EC_{50} values were obtained from concentration-effect curves fitted to data obtained from multiple segments from the same artery (Curves 2 and 3). For some segments, no clear maximum response was observed and it was not possible to calculate $E_{\max}$ or EC_{50} values using non-linear regression analysis, therefore for these segments * denotes the size of the contractile response observed at the highest concentration of agonist tested (100 μM). In addition, estimates were made for the overall $E_{\max}$ and EC_{50} values by fitting concentration-effect curves to the overall mean data obtained in Study 1 and Study 2 and the values shown represent mean and asymptotic s.e. 'n' refers to the number of individual arteries tested. NT=not tested

<i>Patient number</i>	<i>EC</i> ₅₀	<i>E</i> _{max}	<i>EC</i> ₅₀	<i>E</i> _{max}	<i>EC</i> ₅₀	<i>E</i> _{max}
<i>Study 1</i>	<i>5-HT</i>		<i>Sumatriptan</i>		<i>Rizatriptan</i>	
1	0.16	107.2	1.20	28.2	NT	NT
2	0.60	98.9	0.39	158.8	1.32	58.5
4	0.06	118.1	0.23	40.7	0.56	26.5
5	0.75	81.5	—	18.6*	—	1.4*
6	0.11	138.8	0.28	92.8	0.43	74.0
7	1.21	64.3	—	20.6*	2.52	8.3
19	0.36	98.4	3.09	42.0	—	32.7*
Overall value mean	0.22	102.0	0.63	43.7	1.02	22.2
(asymptotic s.e.)	(0.20–0.24)	(1.5)	(0.5–0.7)	(1.8)	(0.8–1.3)	(1.8)
	<i>n</i> = 7		<i>n</i> = 7		<i>n</i> = 6	
<i>Study 2</i>	<i>5-HT</i>		<i>Sumatriptan</i>		<i>L-741,519</i>	
9	0.33	148.6	0.35	82.7	0.13	66.4
10	0.31	146.1	0.59	89.5	0.13	62.4
11	0.40	87.4	—	17.8*	—	8.9*
12	0.85	62.3	—	8.8*	0.16	3.1
13	0.05	119.1	1.81	34.4	0.13	51.7
16	1.24	186.3	3.43	45.8	0.98	19.7
17	0.18	151.1	0.95	38.0	0.11	35.4
18	0.20	126.3	1.47	144.4	0.58	122.3
Overall value mean	0.31	133.8	0.69	57.7	0.09	44.8
(asymptotic s.e.)	(0.3–0.4)	(3.1)	(0.3–1.9)	(0.01)	(0.07–0.13)	(1.2)
	<i>n</i> = 8		<i>n</i> = 8		<i>n</i> = 8	

values for 5-HT and sumatriptan obtained in Study 1 and obtained in Study 2 ($t=9.26$, d.f. = 12; $P<0.001$ and $t=8.01$, d.f. = 12; $P<0.001$ for 5-HT and sumatriptan respectively).

Discussion

In the present experiments, a modified cross-over design (with respect to the order of application rizatriptan and sumatriptan or L-741,519 and sumatriptan to individual arterial strips) was employed to compare directly the effects of rizatriptan and sumatriptan or L-741,519 and sumatriptan on the same segment of human isolated coronary artery. Statistical comparisons showed there was significant desensitization to the agonists throughout the course of the experiment, however, there was no evidence of interaction between this and the measured agonist responses. Therefore, although desensitization did occur, it did not significantly influence comparison of the effects of the 5-HT_{1D}-receptor agonists. Thus desensitization was not considered to exert any influence on the outcome of the investigation.

Variability in 5-HT responsiveness

In human isolated coronary artery, contractile responses to 5-HT were similar in magnitude to contractions evoked by 45 mM KCl and all viable arteries responded to 5-HT. Statistical analysis showed variability in 5-HT responsiveness between arteries obtained from different patients and furthermore, the variability between segments from the same artery differed significantly from patient to patient. In addition there was a significant difference in 5-HT responsiveness between the two studies, where the magnitude of the contractions was greater in Study 2 than Study 1. This probably reflects the smaller magnitude of phasic contractile responses which were often seen in Study 1 but not in Study 2.

Variability of 5-HT responsiveness of human coronary artery is well documented but the underlying reason is not clear. 5-HT responsiveness may be influenced by disease state and in particular atheroma [5, 20] although Kaumann *et al.* [21] reported 5-HT responsiveness was not systematically influenced by disease state or absence/presence of atheroma. Patient studies using angiographic techniques have shown vasodilation of normal coronary arteries in response to 5-HT [3, 4] whereas in patients

Figure 5 The upper panel shows concentration-effect curves to 5-HT (closed squares, $n=7$ arteries), sumatriptan (closed circles, $n=7$ arteries) and rizatriptan (open squares, $n=6$ arteries) obtained in Study 1. The lower panel shows concentration-effect curves to 5-HT (closed squares, $n=8$ arteries), sumatriptan (closed circles, $n=8$ arteries) and L-741,519 (open triangles, $n=8$ arteries) obtained in Study 2. Each point represent overall mean values obtained from all data collected for each agonist in Curves 2 and 3. Vertical bars signify $\pm$ s.e. mean. Curves were fitted to overall mean data using weighted least squares non-linear regression analysis.

with atherosclerotic lesions or in patients with angina, 5-HT causes coronary vasoconstriction [3, 4].

Previously, Ferro *et al.* [17] reported that only 10 out of 22 arteries tested responded to 5-HT. The reason for the difference in the proportion of arteries which responded to 5-HT in the present study and that by Ferro *et al.* [17] is not fully understood but it may reflect differences in experimental methodology or patient population.

Comparison of the effects of 5-HT_{1D}-receptor agonists relative to 5-HT

In the present study, heterogeneity of responsiveness of individual arterial segments to 5-HT_{1D}-agonists (relative to 5-HT itself) was seen. Arterial segments may respond to 5-HT and yet show poor responses to 5-HT_{1D}-

receptor agonists because the effects of 5-HT are mediated, not solely by activation of 5-HT_{1D}-receptors, but by co-activation of other 5-HT receptor subtypes, principally 5-HT₂-receptors. Indeed, it has been reported that 5-HT evoked contractions of human coronary artery can be mediated via activation of both 5-HT₂- and 5-HT_{1D}-receptors [2]. In addition, Kaumann *et al.* [21] have reported variability between different arteries in the contribution of 5-HT_{1D}- and 5-HT₂-receptor activation, showing that for arteries in which 5-HT-induced contractions are mediated predominantly via 5-HT_{1D}-receptors, sumatriptan gives a similar maximum contraction to that produced by 5-HT.

Comparison of the effects of 5-HT_{1D}-receptor agonists

Comparison of the overall EC₅₀ values showed rizatriptan and sumatriptan were similarly potent in causing contraction of human isolated coronary artery, whereas L-741,519 was approximately 10-fold more potent. This observation is consistent with the relative affinities of these compounds for 5-HT_{1D}-receptors measured in radioligand binding studies [13–15] and with the relative potencies in causing contraction of rabbit isolated saphenous vein (a 5-HT_{1D}-receptor bioassay [15, 22, 23]).

Comparison of the overall E_{max} values showed a graded efficacy in the ability of 5-HT_{1D}-receptor agonists to contract human isolated coronary artery, with L-741,519 being less effective than sumatriptan and rizatriptan being the least effective. Thus the present study confirms the conclusion made by Ferro *et al.* [17] that the maximum contractile response evoked by rizatriptan was lower than that for sumatriptan. One of the major differences between the present study and that of Ferro *et al.* [17] was study design. In the present study, direct comparison of the effects of sumatriptan and rizatriptan were made on the same arterial segments (using a cross-over design) whereas in the study by Ferro *et al.* [17] each arterial segment was exposed to a single agonist (sumatriptan or rizatriptan).

The reason why rizatriptan should be less efficacious than other compounds of the same structural class is as yet not known. *In vitro* functional assays using rabbit isolated saphenous vein have shown that rizatriptan, L-741,519 and sumatriptan, are full agonists with respect to 5-HT [15, 22, 23]. Kaumann *et al.* [18, 21] proposed that contractile responses of human coronary and cerebral arteries are mediated via activation of 5-HT_{1DB}-receptors (based on the relative insensitivity of this response to blockade by ketanserin). It is possible that rizatriptan, L-741,519 and sumatriptan have different affinities and/or efficacies at different subtypes of the 5-HT_{1D}-receptor. Alternatively, the lower efficacy of rizatriptan, relative to other 5-HT_{1D}-agonists of the same structural class, may reflect their actions at other members of the 5-HT receptor family. Such speculation should be clarified by molecular biological and radioligand binding studies, and their correlation with pharmacological responses.

There was heterogeneity in the responsiveness of

coronary artery to the 5-HT_{1D}-receptor agonists. For example, for the artery obtained from Patient 2, the E_{max} for sumatriptan was 159% (relative to 45 mM KCl) compared with 59% obtained for rizatriptan, whereas for arteries obtained from Patient 6 and Patient 7 the E_{max} values for sumatriptan and rizatriptan were similar. In the study by Ferro *et al.* [17] heterogeneity of responsiveness to 5-HT_{1D}-receptor agonists was also observed: two out of 10 arteries which responded to 5-HT and sumatriptan showed no contractile response to rizatriptan, and on the other hand one artery responded to 5-HT and rizatriptan but failed to respond to sumatriptan.

Clinical relevance of coronary artery vasoconstriction in migraine therapy

As already discussed by Ferro *et al.* [17] the concentrations of sumatriptan required to cause contraction of isolated coronary arterial segments are higher than the plasma levels observed in the treatment of migraineurs. Thus the clinical relevance of the effects of relatively high concentrations of 5-HT_{1D}-receptor agonists on human isolated coronary artery remains to be established.

In conclusion, in the present experiments on human isolated coronary artery, rizatriptan produced a smaller maximum contraction than sumatriptan, supporting the conclusion made by Ferro *et al.* [17]. Furthermore, the novel 5-HT_{1D}-receptor agonist L-741,519 produced a maximum response intermediate between that of rizatriptan and sumatriptan. The reason for these differences in the effects of structurally-related 5-HT_{1D}-receptor agonists is unclear. The present experiments (in addition to using a different experimental protocol) were conducted in a second experimental centre in a different country and used a different patient population source. Yet the overall conclusions were the same as those of Ferro *et al.* [17]. Therefore, the differences in the effects of 5-HT_{1D}-receptor agonists are undoubtedly real and are worthy of further investigation.

We thank the cardiac surgeons at Baylor Medical College for the supply of tissues and A. Reeve and L. Street for synthesis of sumatriptan, MK-462 and L-741,519.

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(Received 15 November 1995,
accepted 28 March 1996)