Effect of the thromboxane A₂-mimetic U46619 on 5-HT₁-like and 5-HT₂ receptor-mediated contraction of the rabbit isolated femoral artery

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1 The influence of the thromboxane A₂-mimetic U46619 (11 α ,9 α -epoxymethano PGH₂) on 5-hydroxytryptamine (5-HT)-induced contractions of the rabbit isolated femoral artery has been examined.

2 In the absence of U46619, 5-HT responses were mediated predominantly by 5-HT₂-receptors as judged by potent, surmountable antagonism by the selective 5-HT₂ receptor antagonists, spiperone and ketanserin. Both antagonists unmasked a population of 5-HT₁-like receptors which accounted for approximately 10–15% of the 5-HT maximum response.

3 In the presence of U46619 (3–10 nM), 5-HT-induced contractions were largely resistant to blockade by 5-HT₂ receptor antagonists since 5-HT₁-like receptor-mediated contraction now accounted for approximately 60% of the 5-HT maximum response.

4 These results show that activation of thromboxane A₂ receptors in a tissue possessing both 5-HT₂ and 5-HT₁-like receptors can convert 5-HT-induced contraction from one mediated predominantly by 5-HT₂ receptors to one which is mediated predominantly by 5-HT₁-like receptors.

Keywords: 5-HT₁-like receptors; 5-HT₂ receptors; thromboxane A₂; amplification; smooth muscle; rabbit femoral artery; contraction

Introduction

5-Hydroxytryptamine (5-HT) has a long-standing implied role in the pathophysiology of cardiovascular disease involving vasospasm. Such disease states include angina pectoris, cerebral vasospasm which follows subarachnoid haemorrhage, and Raynaud's phenomenon. Evidence for this is provided largely by clinical observations that the concentrations of platelet release products, including 5-HT and thromboxane A₂ (TxA₂), are elevated significantly in patients with vasospastic disease (Robertson *et al.*, 1981; Rubenstein *et al.*, 1981; Van den Berg *et al.*, 1986; Reilly *et al.*, 1986).

Coronary vasospasm, occurring spontaneously or induced by ergonovine, is not prevented by the 5-HT₂ receptor antagonist ketanserin; hence the role of 5-HT in vasospastic angina has been questioned (De Caterina *et al.*, 1984; Freedman *et al.*, 1984). However, it is now apparent that in some human blood vessels, including the umbilical, coronary, basilar and pial arteries, 5-HT can elicit contraction via ketanserin-resistant 5-HT₁-like receptors in addition to ketanserin-sensitive 5-HT₂ receptors (MacLennan *et al.*, 1989; Connor *et al.*, 1989; Parsons *et al.*, 1989; Hamel & Bouchard, 1991). Furthermore, responses mediated by 5-HT₁-like receptors can be 'uncovered' or enhanced following concomitant exposure to other vasoactive agents such as TxA₂ and PGF_{2 α} (Templeton *et al.*, 1991; Sahin-Erdemli *et al.*, 1991). The purpose of this study was to examine the interaction between 5-HT and TxA₂ receptors in the rabbit femoral artery. Initial studies showed that 5-HT-induced contraction of this tissue is mediated predominantly by 5-HT₂ receptors and to a lesser extent by 5-HT₁-like receptors (MacLennan *et al.*, 1991). Using this tissue we sought to investigate how TxA₂ receptor activation might influence the relative contributions made by these receptors to the net response elicited by 5-HT.

Methods

All experiments were conducted on femoral arteries from male New Zealand White rabbits (2.4–3.0 kg) killed by intravenous injection of pentobarbitone sodium (Sagatal: 80 mg kg⁻¹, i.v.) and exsanguination. Ring segments (2.0 mm length) were prepared after first removing adhering connective tissue. The endothelium was removed by gently rolling the tissue around a thin wire. Vascular rings were suspended between two wire hooks (diameter 250 μ m) and immersed in 20 ml organ baths containing Krebs solution (pH 7.4) of the following composition (mM): NaCl 118.41, NaHCO₃ 25.00, KCl 4.75, KH₂PO₄ 1.19, MgSO₄ 1.19, glucose 11.10 and CaCl₂ 2.50. This was maintained at 37°C and continually gassed with 95% O₂/5% CO₂. In all experiments prazosin and mepyramine (both 0.3 μ M) were included in the Krebs solution to exclude actions at α_1 -adrenoceptors and histamine-H₁ receptors respectively.

Experimental protocol

After a 15 min stabilization period, tissues were exposed for 30 min to pargyline (500 μ M) to inactivate monoamine oxidase. During this period each ring segment was set at a passive tension equivalent to 90% of the internal circumference of that artery if it was relaxed and perfused with a transmural pressure of 100 mmHg. For most tissues this tension was 25.5 mN (2.6 g). This procedure is set out in detail elsewhere (Angus *et al.*, 1986) and is an extension of studies in microvessels (Mulvany & Halpern, 1977). Tissues were contracted with KCl (80 mM) to assess tissue viability and provide a reference contracture for subsequent data analysis. Following washout, a single cumulative concentration-effect (E/[A]) curve was constructed either in the absence or presence of antagonists which had been added 60 min previously.

The influence of TxA₂ receptor activation was examined by exposing tissues to the stable mimetic U46619 (11 α ,9 α -epoxymethano PGH₂) to achieve a contraction equal to 30%

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of its maximal effect ($[A_{50}] = 3-10$ nM). $E/[A]$ curves to 5-HT were then constructed in the presence or absence of either spiperone or ketanserin (both $0.3 \mu\text{M}$) which had been added 60 min previously. To characterize further the receptors mediating 5-HT-induced contraction (in the presence of U46619) some tissues were concomitantly exposed to spiperone ($0.3 \mu\text{M}$) and either the 5-HT₃-receptor antagonist MDL72222 ($1.0 \mu\text{M}$) or the non-selective 5-HT₁-like/5-HT₂ receptor antagonist methiothepin (10 nM). The tissues were then exposed to U46619 ($[A_{50}]$) before constructing a single $E/[A]$ curve to 5-HT.

Data analysis

In the absence of antagonists, 5-HT $E/[A]$ curves appeared symmetrical and monophasic and hence the following logistic function was fitted to the data:

$$E = \frac{\alpha \cdot [A]^m}{[A_{50}]^m + [A]^m} \quad (1)$$

in order to estimate the upper asymptote (α), mid-point location ($[A_{50}]$) and the slope parameter (m). Location parameters were actually estimated as logarithms ($-\log_{10}[A_{50}]$). In experiments employing antagonists, 5-HT $E/[A]$ curves were overtly biphasic with a clear-cut inflection. In these experiments only the appropriate antagonist-shifted phase of the 5-HT curve was analysed by equation (1) to provide an estimate of $[A_{50}]$ in the presence of antagonist; from this r , the mid-point concentration-ratio between control and antagonist (B)-treated tissues was calculated. In this way the antagonist dissociation constant (or rather the apparent pK_B) was estimated from the equation:

$$pK_B = \log(r-1) - n \cdot \log[B] \quad (2)$$

where n , the slope of the Schild regression, is unity, i.e. assuming simple competitive antagonism.

Drugs

5-Hydroxytryptamine hydrochloride, prazosin and spiperone were purchased from Sigma (U.K.). U46619 was purchased from Cayman Chemical (U.K.). Mepyramine maleate, methiothepin mesylate, MDL72222 ($1\alpha\text{H}$, 3α , $5\alpha\text{H}$ -tropan-3-yl 3,5-dichlorobenzoate methane sulphonate) and GR32191 ($[1\alpha(Z)$, 2β , 3β , $5\alpha](+)-7-[5-[1,1'-\text{biphenyl}]-4-\text{ylmethoxy}]-3\text{-hydroxy-2(1-piperidinyl) cyclopentyl]-4-heptanoic acid}$) were gifts from Rhone Poulenc (U.K.), Pharmaceutical and Biochemical Research Institute (Czechoslovakia), Marion Merrell-Dow (France) and Glaxo Group Research (U.K.) respectively. All drugs were dissolved and diluted in distilled water with the exception of prazosin and spiperone. Prazosin was dissolved (at 2 mM) in absolute ethanol and diluted in distilled water. Spiperone was dissolved (at 2 mM) in dimethyl sulphoxide (DMSO, 100%) and diluted in distilled water. The final concentrations of vehicle (0.015% v/v) did not influence tissue responsiveness.

Results

5-HT $E/[A]$ curves

In untreated rings of rabbit isolated femoral artery, 5-HT produced apparently monophasic $E/[A]$ curves with a mid-point location ($p[A_{50}]$) of 7.16 ± 0.09 (mean $\pm$ s.e.mean, $n = 4$) and maximum response (relative to 80 mM KCl) of $147 \pm 5\%$ (Figure 1a). However, in the presence of the 5-HT₂ receptor antagonists, spiperone (Figure 1a) or ketanserin (Figure 1b), 5-HT effects became clearly biphasic, revealing a component of the response to lower concentrations of 5-HT which was resistant to 5-HT₂-receptor antagonism. This resistant component comprised approximately 10–15% of the maximum response to 5-HT. Affinity estimates (apparent

Figure 1 Effect of spiperone (a) and ketanserin (b) on concentration-effect ($E/[A]$) curves to 5-HT in the rabbit isolated femoral artery. Responses to 5-HT were obtained in the presence (open symbols) or absence (closed symbols) of U46619. U46619 was added cumulatively to some tissues until a response equal to 30% of its own maximum was achieved ($[A_{50}]$). $E/[A]$ curves to 5-HT were then constructed in control tissues ($\circ$, $\bullet$) or in the presence (Δ , $\blacktriangle$) of spiperone ($0.3 \mu\text{M}$, $n = 4$) or ketanserin ($0.3 \mu\text{M}$, $n = 5$).

pK_B) for spiperone and ketanserin at the receptor mediating the second phase of the effects of 5-HT, calculated from equation (2), were 9.45 ± 0.18 ($n = 4$) and 8.76 ± 0.08 ($n = 5$) respectively. These estimates are similar to pK_B values of 9.28 and 8.56 determined previously at the 5-HT₂ receptor in rabbit aorta (Leff & Martin, 1986).

5-HT was significantly more potent in tissues exposed to U46619 ($p[A_{50}] = 7.99 \pm 0.05$, $n = 4$) than in control tissues ($p[A_{50}] = 7.16 \pm 0.09$) (Figure 1a). Furthermore, in the presence of U46619, 5-HT-induced contractions up to $0.1 \mu\text{M}$ were largely resistant to antagonism by spiperone ($0.3 \mu\text{M}$) and accounted for approximately 60% of the maximum effect. Similar results to those described for spiperone were obtained with ketanserin although there was a less clearly defined plateau between the two phases of the effects of 5-HT (Figure 1b). From visual inspection of the average 5-HT $E/[A]$ curves constructed in the presence of U46619, affinity (apparent pK_B) estimates for spiperone and ketanserin, calculated from the $E/[A]$ curve midpoint concentration-ratios, were 9.35 and 8.57 respectively indicating that antagonism of the second phase of the curve was consistent with 5-HT₂ receptor blockade.

Responses to 5-HT in the presence of spiperone ($0.3 \mu\text{M}$) were analysed further by use of the 5-HT receptor antagonists methiothepin (Figure 2a) and MDL72222 (Figure 2b). The first phase but not the second phase of 5-HT-induced contractions was antagonized by methiothepin (10 nM; $pK_B = 9.24 \pm 0.10$, $n = 6$) whereas neither phase was antagonized by MDL72222 ($1 \mu\text{M}$).

Figure 2 Effect of methiothepin (a) and MDL72222 (b) on concentration-effect ($E/[A]$) curves to 5-HT in the rabbit isolated femoral artery. All responses to 5-HT were obtained in the presence of spiperone ($0.3 \mu\text{M}$). U46619 ($[A_{30}]$) was added to all tissues before constructing $E/[A]$ curves to 5-HT in the absence ($\circ$) or in the presence ($\bullet$) of methiothepin (10 nM , $n = 6$) or MDL72222 ($1.0 \mu\text{M}$, $n = 5$).

U46619 $E/[A]$ curves

$E/[A]$ curves to U46619 were competitively antagonized by the selective TxA_2 receptor antagonist, GR32191 (0.1 – $3.0 \mu\text{M}$); the pK_B was 7.09 ± 0.13 ($n = 4$) and the slope of the Schild plot was 1.12 ± 0.09 (not significantly different from unity). This estimate for GR32191 is similar to the value reported at the TxA_2 receptor in rabbit aorta ($pA_2 = 7.21$, Lumley *et al.*, 1989).

Discussion

The most important observation of this study is that activation of TxA_2 receptors, with low concentrations of the mimetic U46619, converted 5-HT-induced contractions of rabbit femoral artery from being predominantly 5-HT₂ receptor-mediated to being mediated predominantly by 5-HT₁-like receptors. In the absence of TxA_2 receptor activation, 5-HT-induced contractions were largely sensitive to the archetypal 5-HT₂ receptor antagonists spiperone and ketanserin. In the presence of these antagonists, 5-HT $E/[A]$ curves became overtly biphasic revealing a population of receptors resistant to blockade by 5-HT₂ receptor antagonists but which accounted for 10% only of the maximum response. The effect of U46619 was to amplify the phase which was resistant to 5-HT₂ antagonism from representing approximately 10% of the maximum response to 5-HT in control

tissues to approximately 60%. This spiperone- and ketanserin-resistant phase of the effects of 5-HT was potentially antagonized by the 5-HT₁/5-HT₂ receptor antagonist methiothepine ($pK_B = 9.24$) but not by the selective 5-HT₃ receptor antagonist MDL72222 ($1.0 \mu\text{M}$), thus implying involvement of a 5-HT₁-like receptor (Bradley *et al.*, 1986). The high affinity of methiothepin for this receptor is similar to that quoted for the 5-HT₁-like receptor in rabbit saphenous vein ($pK_B = 9.45$, Martin *et al.*, 1991) and suggests that this antagonist has an affinity for vascular 5-HT₁-like receptors in rabbit tissue somewhat greater than reported in other species such as the dog saphenous vein ($pA_2 = 8.26$, Perren *et al.*, 1991), human basilar artery ($pA_2 = 8.80$, Parsons *et al.*, 1989) or guinea-pig iliac artery ($pK_B = 8.07$, Sahin-Erdemli *et al.*, 1991). Furthermore this affinity estimate for methiothepin is considerably higher than for any of the 5-HT₁ binding sites (Hoyer, 1989) suggesting the 5-HT₁-like receptor in rabbit femoral artery may not be identical to any of the 5-HT₁ recognition sites.

Methiothepin (10 nM) did not cause a further shift of the 5-HT₂-receptor-mediated phase of the effects of 5-HT (Figure 2a) which suggests that this antagonist has an affinity lower than 10 nM for the 5-HT₂ receptor in the rabbit femoral artery. In binding experiments methiothepin has a somewhat higher affinity at the 5-HT₂ recognition site ($pK_i = 8.8$, Hoyer, 1989). With one exception, methiothepin does not behave as a competitive antagonist at peripheral 5-HT₂ receptors (see Mylecharane, 1990) and thus rigorous estimates of its affinity are uncommon. In dog femoral artery methiothepin is a competitive antagonist with a pA_2 of 8.80 (Cohen, 1986). Thus methiothepin appears to have a lower affinity for the 5-HT₂ receptor in rabbit femoral artery than in other tissues, although a more rigorous analysis has to be carried out.

Augmentation of 5-HT₁-like receptor-mediated responses in rabbit femoral artery is not exclusive to U46619. Similar augmentation can be achieved with threshold concentrations of angiotensin II and histamine (unpublished observations). Since each of these agents augment 5-HT₁-like responses in a similar manner, it is probable that the transduction pathways associated with their receptors share common elements, which, in some way, leads ultimately to their effects on 5-HT₁-like receptor-mediated effects. The mechanism(s) underlying this augmentation are presently under investigation.

It is apparent from our results that the maximum response to 5-HT is not changed by U46619, implying that 5-HT₂ receptor-mediated effects are not augmented. We have previously examined the interaction between TxA_2 and 5-HT₂ receptors in more detail (MacLennan *et al.*, 1991). U46619 is able to potentiate the effects of ($\pm$)- α -methyl-5-HT acting at 5-HT₂ receptors but this potentiation is characterized by a small leftward shift only of ($\pm$)- α -methyl-5-HT curves (less than three fold) without an increase of the maximum response. Thus, with respect to 5-HT receptors in the rabbit femoral artery, U46619 exclusively augments 5-HT₁-like mediated effects.

The ability of U46619 to augment 5-HT₁-like responses is directly related to occupancy of TxA_2 receptors rather than to any non-specific action since in tissues exposed to U46619 (EC_{30}) and in the presence of GR32191 ($3 \mu\text{M}$) such that no contraction is observed, 5-HT₁-like receptor-mediated responses are not augmented (unpublished observations).

From our observations in the rabbit femoral artery it is intriguing to speculate whether such amplification of 5-HT₁-like responses occurs in other vascular smooth muscle tissues such as the human coronary artery in which 5-HT₁-like receptors normally contribute minimally to the total effects of 5-HT (Connor *et al.*, 1989). The concentrations of both 5-HT and TxA_2 released presumably from aggregating platelets, are elevated in the coronary sinus blood of patients with angina involving coronary artery disease and vasospasm (Robertson *et al.*, 1981; Van den Berg *et al.*, 1989). In this scenario it

would be anticipated from our results that 5-HT₁-like receptor-mediated contraction would be augmented and thus provide an explanation for the failure of 5-HT₂ receptor

antagonists such as ketanserin to relieve vasospastic angina (De Caterina *et al.*, 1984; Freedman *et al.*, 1984).

We would like to thank Mary Bolofo for excellent technical assistance.

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(Received April 9, 1992)

Revised May 29, 1992

Accepted June 4, 1992)