Interaction Between Thromboxane A2 and 5-Hydroxytryptamine Receptor Subtypes in Human Coronary Arteries

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Background. Platelets release two powerful vasoconstrictors—thromboxane A2 (TXA2) and 5-hydroxytryptamine (5-HT). Animal studies have suggested that these two substances may act in a synergistic fashion to stimulate platelet activity and smooth muscle vasoconstriction.

Methods and Results. To assess the interaction between TXA2 and 5-HT, at a minimal concentration of U46619. A total of 168 vessel segments were removed from 20 recipient hearts of patients undergoing cardiac transplantation. Segments were set up in isolated organ baths and tested for their response to 5-HT in the presence of an EC50, EC80, or EC90 concentration of U46619 (n=4). A synergistic response was only seen in a small number of the segments tested under these conditions. However, in the presence of ketanserin (10^-6 M) to block 5-HT2 receptors, there was a significant increase in the response to 10^-5 M 5-HT in the presence of both the EC50 (p<0.025) and EC80 (p<0.05) concentrations of U46619 (n=4). The potentiation of non-5-HT1 receptor mediated responses to 5-HT, in the presence of U46619 (EC80), could be prevented by 10^-7 M methiothepin, a nonselective 5-HT1-like/5-HT2 receptor antagonist.

Conclusions. These data indicate that TXA2 receptor activation can increase the response of 5-HT mediated by 5-HT1-like receptors in human coronary arteries. 5-HT1-like receptors have been shown to mediate the contractile effect of 5-HT in patients with variant and chronic stable angina. Thus, platelet contents may act together at specific receptor subtypes in the induction of myocardial ischemia. (Circulation 1993;87:874-880)

Key Words • serotonin • platelets • synergy • coronary vasospasm • U46619

Platelet disruption has been shown to release the powerful vasoconstrictor substances, 5-hydroxytryptamine (5-HT) and thromboxane A2 (TXA2). Both of these substances are candidates for the mediation of vasospasm since they are found in increased concentrations at the site of coronary arterial stenosis in animal models of vasospasm^2^ and in patients with acute coronary syndromes.5,6

Furthermore, it has been shown that the response to intracoronary injection of 5-HT is converted from a mild dilatation in healthy coronary arteries to a vasoconstriction in patients with atherosclerosis,6,7 but the receptor subtypes mediating this response have not been adequately characterized. Recent in vivo and in vitro studies suggest a possible role for 5-HT1-like and 5-HT2 receptors in mediating 5-HT-induced constriction in patients with ischemic heart disease.8-10

The concentration of 5-HT and TXA2 that confronts the arterial wall following platelet disruption in vivo is difficult to judge. The vasoconstrictor effect of aggregating platelets may rely on the action of either 5-HT or TXA2 alone or an interaction between the two. Such an interaction of coreleased substances may be mediated through receptor "cross-talk," resulting in additive or synergistic effects. A synergistic action of 5-HT and TXA2 has been demonstrated in the induction of cyclic flow variations in coronary flow in dog coronary arteries in vivo.11

The aim of this study was to investigate whether such amplifying interactions between 5-HT and TXA2 receptors may occur in human coronary arteries and to assess the role of the different subtypes of 5-HT receptor in the interaction between these two vasoconstrictors.

Methods

A total of 168 vessel segments of epicardial coronary arteries were removed from 20 patients (mean age, 27 years) at the time of cardiac transplantation. These patients had been diagnosed as having angiographically normal coronary arteries and were undergoing transplantation for conditions other than ischemic heart disease (i.e., cardiomyopathy, congenital heart defects, lung disorders). These arteries showed no signs of atheroma on gross examination or under a dissecting microscope. This was confirmed in a selection of segments via histological examination. Arteries were placed immediately in a modified Tyrode's solution of

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FIGURE 1. Comparison of the contractile effect of U46619 (filled circles, n=4) and 5-HT (open circles, n=4) on healthy human epicardial coronary arteries. The graph also shows the calculation of EC10, EC30, and EC50 values for U46619.

the following composition (mM): NaCl 136.9, NaHCO3 11.9, KCl 2.7, NaH2PO4 0.4, MgCl2 2.5, CaCl2 2.5, glucose 11.1, and disodium EDTA 0.04.

Segments (3–5 mm in length) of epicardial coronary artery were mounted between two metal hooks in organ baths containing the modified Tyrode’s solution, which was maintained at 37°C while being gassed with 95% O2-5% CO2. Each segment was given an initial pretension of 50 mN, which was allowed to relax over a period of 60–90 minutes. This was followed by addition of 90 mM KCl. Once a stable contraction had been attained, the KCl was removed by changing the bath Tyrode’s twice. When a stable baseline had been reestablished, the KCl depolarization was repeated. This was done to

FIGURE 2. Contractile effect of 5-HT in the absence of U46619 (open circles, n=4) and the presence of the EC10 (filled circles, n=4), EC30 (open triangles, n=4), and EC50 (filled triangles, n=4) concentrations of U46619 on healthy epicardial coronary arteries.
TABLE 1. Maximum Effect and EC₅₀ Values of 5-HT After Subtraction of Effect of U46619

<table>
<thead>
<tr>
<th>Concentration of U46619</th>
<th>Maximum effect of 5-HT (%90 mM KCl)</th>
<th>EC₅₀ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>65.1±8.0</td>
<td>1.9±0.5×10⁻⁷</td>
</tr>
<tr>
<td>EC₁₀</td>
<td>56.5±7.8</td>
<td>1.1±0.2×10⁻⁷</td>
</tr>
<tr>
<td>EC₃₀</td>
<td>73.8±12.7</td>
<td>8.9±2.8×10⁻⁸</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>40.0±8.9</td>
<td>1.3±0.5×10⁻⁷</td>
</tr>
</tbody>
</table>

5-HT, 5-hydroxytryptamine.

assess tissue viability and to attain consistent responses from each vessel segment.

In a preliminary series of experiments, the dose–response relation of the thromboxane mimetic U46619 and 5-HT were obtained so that the EC₁₀, EC₃₀, and EC₅₀ values for U46619 or 5-HT could be calculated (the EC value represents the effective concentration that achieves 10%, 30%, and 50% of the maximum effect).

Once a stable baseline tension had been achieved after the second KCl depolarization, tissues were exposed to an EC₁₀, EC₃₀, or EC₅₀ concentration of U46619. These concentrations were allowed to develop their maximum tension; once a plateau had been attained, increasing concentrations of 5-HT were added to the baths in ½log₁₀ units, while the U46619 was still present. Additions of 5-HT were made until the maximum response of 5-HT had been reached. Some vessel segments received no U46619 before the addition of 5-HT and acted as controls.

In a second series of experiments, vessel segments were incubated with the 5-HT₂ receptor antagonist ketanserin (10⁻⁶ M) for 1 hour after the second KCl response. Tissues were then exposed to a single dose of 5-HT (10⁻⁶ M), which corresponded approximately to the EC₀ concentration of 5-HT in the presence of 5-HT₂ receptor blockade. Once this dose of 5-HT had produced a stable contraction of the tissue, it was removed by changing the bath Tyrode’s twice. When baseline conditions had returned, the tissues were then exposed to an EC₁₀, EC₃₀, or EC₅₀ concentration of U46619 and allowed to reach a stable level of contraction, at which point the same dose of 5-HT was applied.

**FIGURE 3.** Recordings of responses obtained from segments of epicardial coronary artery from (left panel) male, 31 years, transplanted for dilated cardiomyopathy, and (right panel) female, 46 years, transplanted for dilated cardiomyopathy. All artery segments were free of atherosclerotic lesions. The responses to 5-HT are potentiated in the presence of either the EC₁₀ or EC₃₀ of U46619. The graphs show the mean responses of the segments from each patient (two segments per curve).
5-HT corresponded to 65.0±8.8% of the response induced by 90 mM KCl, whereas that of U46619 was 180.2±7.9%. The EC_{50} concentrations for 5-HT and U46619 were 1.9±0.5×10^{-7} and 7.5±1.3×10^{-9} M, respectively.

The concentrations of U46619 corresponding to the EC_{10}, EC_{30}, and EC_{50} were interpolated from the individual dose–response curves with mean values of 1.9±0.3×10^{-6} M (EC_{10}), 4.0±1.1×10^{-6} M (EC_{30}), and 7.5±1.3×10^{-9} M (EC_{50}). These concentrations of U46619 were used in the subsequent parts of the study.

Segments of healthy coronary artery that had been exposed to an EC_{10}, EC_{30}, or EC_{50} concentration of U46619 all developed an active tension. 5-HT was able to elicit further concentration-related contractions in tissues exposed to all three concentrations of the TXA_{2} mimetic (Figure 2). The maximum effect achieved by 5-HT in these tissues (after subtraction of the U46619 effect) decreased in the order EC_{30}>control>EC_{10}>EC_{50} (Table 1). However, the maximum effect of 5-HT at any level of U46619-induced tone was not statistically different from the control (no U46619). The presentation of the mean data masks important changes in the reactivity to 5-HT observed in some tissues.

In artery segments from two patients, it was possible to see either an increase in potency and/or an increase in the maximum effect of 5-HT in the presence of the thromboxane mimetic. Representative traces and graphs from these patients are shown in Figure 3. The induction of cyclic activity on induced constriction was common to segments that received the higher doses of U46619. Usually, 5-HT was still capable of inducing further increases in tension that was underlying this cyclic activity.

In a second series of experiments, the interaction between 5-HT (10^{-6} M) and U46619 was examined in the presence of ketanserin (10^{-6} M) to block the effect mediated by 5-HT_{1} receptors. The effects of 5-HT were augmented in the presence of U46619, and this increase was related to the concentration of U46619 present (Figure 4). The total maximum (5-HT and U46619) responses were similar for the EC_{10} and EC_{30} concentrations of U46619. The mean 5-HT responses were significantly higher in the presence of the EC_{10} and EC_{30} concentrations of U46619 compared with controls (Figure 5).

The potentiated response to 5-HT (in the presence of ketanserin) by U46619 (EC_{50}) was prevented by the additional incubation of the vessel segments with 10^{-7} M methiothepin (Figure 6). The presence of methiothepin, a nonselective 5-HT_{1}-like/5-HT_{2} antagonist, abolished the significant increase in the response to 5-HT.

**Discussion**

The present study has demonstrated a synergistic action between the thromboxane mimetic and 5-HT in human coronary arteries. However, this synergy appears to occur preferentially at non-5-HT_{1} receptors. Two pieces of evidence support this conclusion. First, the maximal effect of 5-HT was not significantly increased by U46619. Second, in the presence of ketanserin, which blocked the 5-HT_{1} receptors, the small remaining effect of 5-HT was significantly augmented by U46619. This augmentation was blocked by further addition of the nonselective 5-HT_{1}-like/5-HT_{2} receptor...
antagonist methiothepin. These results imply that 5-HT₁-like receptor-mediated effects can be preferentially augmented by U46619.

The mixed effects seen with 5-HT and U46619 (in the absence of ketanserin) are probably a reflection of the heterogeneous population of 5-HT receptors that mediate contraction in human epicardial coronary arteries. Only approximately 30% of the response to 5-HT is mediated via 5-HT₁-like receptors and is therefore generally masked by the predominant 5-HT₂ receptor-mediated contraction. Thus, averaging the data overrids much of the effects mediated by non-5-HT₂ receptors. Despite this, closer examination of individual responses shows that the contraction to 5-HT can be augmented by U46619 in some vessel segments, which may be a reflection of variations in the function and/or expression of 5-HT₁-like receptors.

Interactions between TXA₂ and 5-HT have previously been shown to enhance the activity of platelets and to augment their biological effects. The formation of TXA₂ has also been shown to enhance the release of 5-HT from storage sites in the dense tubular system of platelets. Inhibition of platelet-induced cyclic flow variations in dogs has been shown to require a combination of both 5-HT₂ receptor and TXA₂ receptor antagonists. In a similar manner, the efficacy of thrombolysis is enhanced by simultaneous administration of TXA₂ and 5-HT₂ receptor antagonists in a canine model.

The interaction between 5-HT and U46619 has important consequences for the potential action of 5-HT in the mediation of vasospastic events. It increases the functional relevance of non-5-HT₂ receptors when stimulated by 5-HT in the presence of TXA₂. This may have important clinical implications since it has previously been demonstrated that 5-HT₁-like receptors are preserved in regions distal to atherosclerotic lesions in diseased coronary arteries. This region in which 5-HT₁-like receptors appear to be preserved is the same area in which vasoconstriction induced by platelets has been shown to occur, i.e., areas immediately distal to stenosis. In addition, these areas of stenosis represent sites where platelet aggregation and disruption are maximal and thus could result in marked elevation of platelet-derived vasoactive mediators.

Platelet-induced vasoconstriction of isolated bovine coronary arteries has been shown to be due to two separate mechanisms. The first phase, which is rapid in onset and of short duration, is thought to be due to TXA₂, whereas the second phase, which is slower in onset but long lasting, is mediated by 5-HT. The data in the present study indicate that the magnitude of the 5-HT-mediated phase of platelet-induced vasoconstriction may be enhanced by the prior release of TXA₂.

Our study has shown that none of the vessels precontracted by U46619 showed any relaxation in response to 5-HT. The integrity of the endothelium in such segments is maintained, as reported in our previous studies. This confirms previous reports that human epicardial coronary arteries do not have a direct relaxant response to 5-HT and that the small relaxation to 5-HT seen in vivo probably occurs via an indirect mechanism.

There is increasing evidence that platelet-derived 5-HT is involved in the mediation of vasospastic events. 5-HT has been shown to be present in increased concentrations in patients with limiting angina and coronary lesions. Furthermore, this amine has divergent effects in patients with atherosclerotic compared with healthy arteries. In vivo and in vitro studies...
have demonstrated that regions distal to atherosclerotic lesions, a region in which vasospasm usually occurs, have ketanserin-resistant 5-HT$_1$-like receptors. These 5-HT$_1$-like receptors mediated 50% of the total response of 5-HT in diseased arteries, whereas they were capable of inducing a contraction equal to 30% of the maximum achieved by 5-HT in healthy arteries.

The failure of ketanserin in the treatment of coronary vasospasm has cast doubt on the role of 5-HT receptors in the mediation of vasospasm. However, this antagonist is ineffective at blocking the action of 5-HT at 5-HT$_1$-like receptors. Our results show that 5-HT-induced constriction mediated by 5-HT$_1$-like receptors can be augmented after activation of TXA$_2$ receptors. The possibility exists that there are areas in diseased coronary arteries where the effect mediated by these receptors may be sufficient to cause coronary occlusion. Further studies are warranted that use more specific receptor probes. The use of the selective 5-HT$_1$-like receptor agonists and the development of a specific antagonist at the 5-HT$_1$-like receptor will permit clarification of the combined role of these receptors in coronary vasospasm in vivo.

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References


Figure 6. Effect of methiothepin (10$^{-7}$ M) on the interaction between U46619 (EC$_{30}$) and 5-HT (10$^{-6}$ M) in the presence of ketanserin (10$^{-6}$ M). The open bar shows the response to 5-HT alone (n=4), the cross-hatched bar shows the response of an identical dose of 5-HT in the presence of the EC$_{30}$ concentration of U46619 (n=4), and the filled bar shows the effect of 30 minutes' incubation with methiothepin (10$^{-7}$ M) on the response of 5-HT in the presence of the thromboxane mimetic (n=4). All responses were obtained in the presence of ketanserin (10$^{-6}$ M).

NS, nonsignificant compared with pre-U46619 response; *p<0.05 compared with pre-U46619 response.


