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Combined administration of 5-HT_2 and thromboxane A_2 antagonists: effects on platelet aggregation and isolated cardiac muscle

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1 To investigate possible mechanisms underlying the ability of combined administration of a 5-hydroxytryptamine$_2$ (5-HT_2) antagonist and a thromboxane A_2 antagonist to reduce reperfusion-induced arrhythmias, the effects of these drugs alone and in combination on platelet aggregation and on cardiac muscle were determined.

2 Platelet aggregation was measured in whole blood obtained from anaesthetized rats. Concentrations of 5-HT (10 μM) and the thromboxane A_2 mimetic U46619 (1 μM) which did not cause aggregation themselves, enhanced the responses to ADP (0.1 μM) and to collagen (1 μg ml^{-1}). For example, the response of 1.0 ± 0.5 Ω to ADP alone was increased significantly to 6.4 ± 1.0 Ω by 5-HT, 15.5 ± 2.8 Ω by U46619, and 17.3 ± 1.3 Ω when U46619, 5-HT and ADP were added together.

3 In further experiments blood was obtained from rats which had received either the 5-HT_2 antagonist, ICI 170,809 (1 mg kg^{-1}), or the thromboxane A_2 antagonist, ICI 192,605 (1 mg kg^{-1} min^{-1}), or both in combination. When ADP was used as the primary aggregating agent, the ability of U46619 alone, or together with 5-HT, to enhance responses was reduced significantly by ICI 192,605 alone and in combination with ICI 170,809. Similar results were obtained with lower doses of ICI 170,809 (0.3 mg kg^{-1}) and ICI 192,605 (0.3 mg kg^{-1} min^{-1}).

4 When collagen was used as the primary aggregating agent ICI 170,809 (1 mg kg^{-1}) reduced the response to 5-HT (5.0 ± 0.8 Ω versus 10.9 ± 1.2 Ω in controls), and ICI 192,605 (1 mg kg^{-1} min^{-1}) reduced the response to U46619 (6.8 ± 2.5 Ω versus 11.2 ± 2.2 Ω in control). The greatest reduction of platelet aggregation was seen in blood from rats which had received both antagonists, with the response to U46619 plus 5-HT plus collagen being 2.7 ± 0.6 Ω compared to 14.2 ± 1.7 Ω in controls. In contrast, there was no significant attenuation of platelet aggregation in blood from rats which had received the lower doses of each antagonist alone. Only the combination of ICI 170,809 (0.3 mg kg^{-1}) and ICI 192,605 (0.3 mg kg^{-1} min^{-1}) reduced the response to U46619 plus 5-HT plus collagen (7.6 ± 1.4 Ω versus 15.0 ± 0.5 Ω in controls).

5 In rat isolated ventricular muscle preparations, ICI 170,809 increased the effective refractory period; e.g. from 39 ± 4 to 86 ± 18 ms, 10 min after adding 30 μM to left papillary muscles. ICI 192,605 did not increase the effective refractory period itself and did not alter the ability of ICI 170,809 to prolong the effective refractory period. In the presence of 100 μM ICI 192,605, ICI 170,809 (30 μM) increased the effective refractory period from 38 ± 7 to 100 ± 30 ms.

6 These results indicate that the previously observed antiarrhythmic activity of combined administration of the higher doses of ICI 170,809 and ICI 192,605 is unlikely to be due to direct effects on cardiac muscle but could be a consequence of reduced platelet aggregation.

Keywords: 5-Hydroxytryptamine; thromboxane A_2; U46619; platelet aggregation; effective refractory period; cardiac muscle; ICI 170,809; ICI 192,605; 5-HT_2 antagonist; TP antagonist

Introduction

Previously, it has been shown that combined administration of a 5-hydroxytryptamine$_2$ (5-HT_2) antagonist and a thromboxane A_2 antagonist reduced reperfusion-induced arrhythmias, but not ischaemia-induced arrhythmias, in anaesthetized rat models of coronary artery occlusion and reperfusion (Shaw & Coker, 1996a). It was only when both drugs, the 5-HT_2 antagonist ICI 170,809 and the thromboxane A_2 antagonist ICI 192,605, were given together that there was marked suppression of reperfusion-induced ventricular tachycardia and ventricular fibrillation. Neither of the drugs given alone significantly altered the incidence of these arrhythmias. The mechanism(s) responsible for the enhanced effectiveness of this drug combination against reperfusion-induced arrhythmias remain to be elucidated.

Both thromboxane A_2 and 5-hydroxytryptamine (5-HT) are major products of platelet aggregation (Steen & Holmsen, 1987) which cause vasoconstriction and can induce or enhance further platelet aggregation (De Clerck & Herman, 1993; Siess, 1989). Interactions between 5-HT and thromboxane A_2 have been implicated in models of coronary thrombosis, e.g. the development of cyclical flow variations in a canine model of severe coronary artery stenosis (Ashton *et al*., 1987). It has also been shown that combined administration of a 5-HT_2 antagonist and a thromboxane A_2 antagonist enhanced thrombolysis with streptokinase (Vandeplassche *et al*., 1993). Decreased platelet aggregation has been suggested as an explanation for these effects in models, where events are dependent upon reperfusion of occluded coronary arteries. Thus, it is possible that reductions in platelet aggregation may explain the significant antiarrhythmic activity of the combination of 5-HT_2 and thromboxane A_2 receptor blockade.

It is also possible however, that the antiarrhythmic activity observed previously could simply be due to direct effects of this particular drug combination on cardiac muscle. The 5-HT_2

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antagonist ICI 170,809 has been shown to prolong the maximum driving frequency in rat isolated atrial and ventricular preparations (Ellis & Coker, 1992). As far as we are aware no information is available about the direct effects of the thromboxane A_2 antagonist ICI 192,605 on cardiac muscle, whether given alone or in combination with other drugs. Thus the aims of the present work were to examine the effects of the 5-HT$_2$ antagonist ICI 170,809 and the thromboxane A_2 antagonist ICI 192,605 alone and in combination on platelet aggregation and to determine their direct effects on cardiac muscle function. Preliminary accounts of some of this work have been presented to the British Pharmacological Society (Shaw & Coker, 1994; Coker & Batey, 1995).

Methods

Platelet aggregation studies

Platelet aggregation was measured by the impedance method in heparin-treated whole blood obtained from anaesthetized rats which had received the drugs under investigation. Two separate studies were performed investigating either the higher or lower doses of the drugs that were used previously in the studies on reperfusion-induced arrhythmias (Shaw & Coker, 1996a). The high dose drug study was performed in the Department of Pharmacology and Therapeutics at the University of Liverpool, with a single channel Chronolog Whole Blood Aggregometer on loan from Labmedics (Stockport). The lower dose drug study was performed at Astra Charnwood (formerly Fisons Pharmaceuticals) in Loughborough, with a double channel Chronolog Whole Blood Aggregometer. Male Wistar rats for the high dose study were obtained from the Nuffield Joint Facilities, The University of Liverpool (weight range 315 to 435 g), whereas those used for the lower dose drug study were obtained from Bantin and Kingman, Hull (weight range 395–500 g).

Rats were anaesthetized and the trachea, a carotid artery and a femoral vein were cannulated in preparation for ECG and blood pressure monitoring as described previously for studies on ischaemia-induced and reperfusion-induced arrhythmias (Barnes & Coker, 1995; Shaw & Coker, 1996a). A left thoracotomy was carried out and the rat ventilated as before. Arterial blood gases and pH were monitored and the ventilation volume adjusted if necessary to maintain values within normal limits. The heart was exteriorised briefly to mimic the arrhythmia studies. Drug or vehicle administration commenced and 15 min later blood was removed via the arterial cannula and placed in a tube containing 50 u ml^{-1} heparin. A 1 ml arterial blood sample was put into a potassium EDTA tube for platelet counting. Platelet counts were carried out at the Royal Liverpool University Hospital Haematology Department in a Coulter STKS machine. In the lower dose drug study ECG, haemodynamics, blood gases and platelet counts were not measured as suitable equipment was not available.

Blood for platelet aggregation was dispensed in 0.45 ml aliquots into siliconized cuvettes containing 0.55 ml of isotonic saline and the samples were warmed to 37°C. Platelet aggregation was measured by the increase in impedance across electrodes to which platelets and platelet aggregates adhere (Cardinal & Flower, 1980). After an equilibration period, aggregation to adenosine 5′-diphosphate (ADP) 0.1 μM and to collagen 1 μg ml^{-1} was determined. The abilities of 5-HT and U46619 (a thromboxane mimetic) alone or together, to potentiate ADP- and collagen-induced aggregation were also examined. U46619 (1 μM) was added 1 min before ADP or collagen and 5-HT (10 μM) was added 15 s before ADP or collagen. The concentrations of aggregating agents to be used were determined in preliminary studies in blood taken from untreated anaesthetized rats.

The two separate studies each contained four groups of rats with $n=6$ per group. In each study anaesthetized rats were allocated randomly to one of the four groups: control; 5-HT$_2$ antagonist – ICI 170,809; thromboxane antagonist – ICI 192,605; or both drugs. Each rat received a bolus dose (1 ml kg^{-1}) of ICI 170,809 (or its vehicle, acidified water) followed immediately by a continuous infusion (0.05 ml min^{-1}) of ICI 192,605 (or its vehicle, alkaline saline). Thus, rats in the control group received both vehicles, those in the 5-HT$_2$ antagonist group received ICI 170,809 plus the vehicle for ICI 192,605, those in the thromboxane A_2 antagonist group received ICI 192,605 plus the vehicle for ICI 170,809 and the final group received both the drugs. In the first study, the 'high dose study', the 5-HT$_2$ antagonist was administered as a 1 mg kg^{-1} bolus and the thromboxane A_2 antagonist was infused at 1 mg kg^{-1} min^{-1}. In the second study, the 'low dose study', the 5-HT$_2$ antagonist was administered as a 0.3 mg kg^{-1} bolus and the thromboxane A_2 antagonist infused at 0.3 mg kg^{-1} min^{-1}.

Isolated cardiac muscle preparations

Experiments were performed in tissues from male Wistar rats (270–395 g) obtained from the Nuffield Joint Facilities, The University of Liverpool. The rats were treated with heparin (500 u, i.p.) and anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). The thorax was opened and the heart removed rapidly and placed in a Krebs-bicarbonate solution of the following composition (mM): NaCl 119, KCl 3.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10 and $CaCl_2$ 1.9. A right ventricular strip and a left papillary muscle were dissected out rapidly and a thread attached to one end of each tissue. The opposite end of each preparation was impaled on one pole of a bipolar platinum electrode, which was then placed in Krebs solution in a 30 ml organ bath maintained at 37°C and gassed with 95% O_2/5% CO_2. Preparations were suspended under a resting tension of 10 mN and paced at 1 Hz with square wave pulses of 5 ms duration at twice threshold voltage, with Grass S48 or S88 stimulators. Developed tension was measured by Lectromed UF1 isometric force transducers (57 g sensitivity range) connected to 5240 pre-amplifiers and a Lectromed MT6 recorder. Preparations were allowed to equilibrate for 1 h. During this time the preparations were washed 3 to 4 times and the resting tension reset if necessary.

The effective refractory period was measured by a modification of the extra stimuli method of Scholtysik, (1980). At 30 s intervals a series of five extra stimuli were applied. These extra stimuli were delivered at the same pulse width, voltage and frequency as the 1 Hz pacing stimulus, but at a known delay after each normal pacing stimulus. The delay between normal and extra stimuli was increased gradually until an increase in developed tension was noted in response to the extra stimuli. The lowest delay at which this occurred was assumed to be the effective refractory period. Care was taken to check the threshold voltage at regular intervals, and to adjust the stimulation voltage if necessary, to ensure that all measurements of effective refractory period were made at twice threshold stimulation voltage.

After three control measurements of effective refractory period had been obtained at 5 min intervals, the vehicle was added and measurements of effective refractory period were obtained 5, 10 and 15 min later. The preparation was then washed, another measurement of effective refractory period obtained, the first concentration of drug added and its effects measured 5, 10 and 15 min later. This sequence was repeated with increasing concentrations of drug. In each preparation a concentration-response curve was obtained for either the 5-HT$_2$ antagonist ICI 170,809, the thromboxane A_2 antagonist ICI 192,605, or ICI 170,809 in the presence of a fixed concentration of ICI 192,605 (100 μM).

Drugs

ICI 170,809 (2-2[dimethylamino-2-methylpropylthio]-3-phenylquinoline hydrochloride) (Blackburn *et al*., 1988; Millson *et*

al., 1992), also described more recently as ZM170809 (McAuliffe *et al*., 1994) and ICI 192,605 (4(Z)-6-(2,4,5 *cis*)[2-chlorophenyl)-4-(2-hydroxyphenyl) 1,3-dioxan-5-yl]hexenoic acid) (Jessup *et al*., 1988) were gifts from Zeneca Pharmaceuticals (Macclesfield). ICI 170,809 was dissolved in distilled water with 1 M HCl added dropwise until a clear solution was obtained. ICI 192,605 was dissolved in saline with 1 M NaOH added dropwise until a clear solution was obtained. Control vehicle solutions also contained equivalent concentrations of HCl (0.04 N) or NaOH (0.04 N). Sodium pentobarbitone (Sagatal) was obtained from RMB Animal Health Ltd (Dagenham). Heparin (Multiparin, CP Pharmaceuticals Ltd., Wrexham) was obtained through the Royal Liverpool University Hospital Pharmacy. ADP, U46619 (9,11dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$ and 5-HT creatinine sulphate were purchased from Sigma (Poole) and collagen from Labmedics (Stockport). All salts for Krebs solution were purchased from BDH (Poole).

Statistics

Values are expressed as mean ± s.e.mean of n experiments. Between group differences in haemodynamics were compared by one way analysis of variance with subsequent modified t tests (with Bonferroni corrections). Other data that may not be distributed normally were compared by use of Kruskal-Wallis tests with subsequent multiple comparisons where appropriate. A probability of $P<0.05$ was considered to be significant.

Results

Effects of 5-HT and U46619 on platelet aggregation

In the preliminary studies with heparin-treated blood taken from anaesthetized rats which had not received any pretreatment, ADP 1 μM induced an aggregation response of 23.0 ± 2.0 Ω whereas the response to 0.1 μM ADP was only 1.0 ± 0.5 Ω. Optimal potentiation of the response to 0.1 μM ADP by 5-HT was achieved when 10 μM 5-HT was added 15 s before ADP. With the thromboxane mimetic U46619, 1 μM added 1 min before ADP 0.1 μM caused marked potentiation of platelet aggregation. The greatest potentiation of the response to ADP occurred when both 5-HT and U46619 were present (Figure 1). These concentrations of 5-HT and U46619 did not cause any aggregation when given alone, but when given in combination substantial aggregation (17.3 ± 1.3 Ω) occurred in blood from 4 rats with no response in 2 others. These concentrations of U46619 and 5-HT also caused significant potentiation of the platelet aggregation response to collagen 1 μg ml^{-1} (Figure 1).

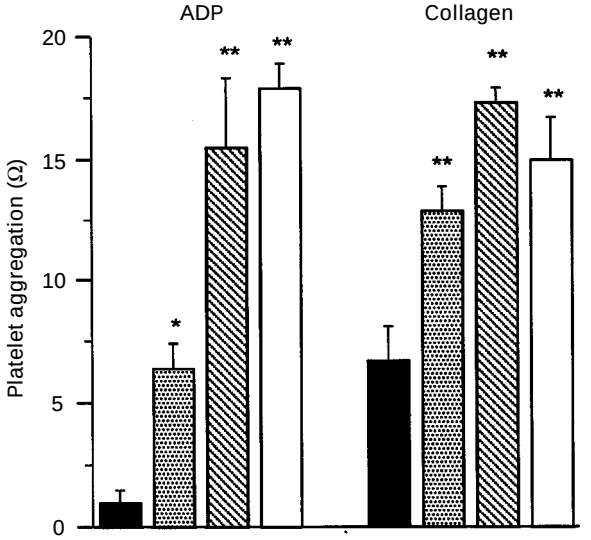

Figure 1 The effects of 5-HT 10 μM (stippled columns) and U46619 1 μM (hatched columns) alone and in combination (open columns) on platelet aggregation induced by ADP 0.1 μM or collagen 1 μg ml^{-1} (solid columns) in rat whole blood. U46619 was added 1 min, and 5-HT 15 s, before the primary aggregating agent (ADP or collagen). Each value is the mean ± s.e.mean, $n=6$. *$P<0.05$, **$P<0.01$ compared to primary aggregating agent alone, Kruskal Wallis test.

Effects of ICI 170,809 and ICI 192,605 on ADP-induced platelet aggregation

In whole blood taken from control rats which had received both vehicles for the high dose drug study, aggregation to ADP 0.1 μM produced a small response of 4.3 ± 0.8 Ω. When 5-HT 10 μM was added 15 s before ADP the response was 6.5 ± 1.0 Ω. In the presence of the thromboxane-mimetic U46619 1 μM the response to ADP was significantly enhanced and combined addition of U46619 10 μM, 5-HT 10 μM and ADP 0.1 μM produced the largest aggregation response (Figure 2). In these experiments, addition of 5-HT 10 μM or U46619 1 μM, either alone or together, did not cause any platelet aggregation. A similar pattern of responses was seen in blood obtained from control rats in the low dose study (Figure 2).

The response to ADP alone was significantly less in the blood from rats that had received the higher doses of both ICI 170,809 and ICI 192,605 compared to the controls, whereas no significant differences were observed between groups in the low dose study (Figure 2a). Although the platelet aggregation response to ADP plus 5-HT appeared to be less in blood from the groups which had received the antagonists, there were no significant differences between the drug-treated groups and the controls in either the low or high dose study (Figure 2b). The ability of U46619 to potentiate responses to ADP was reduced in blood from rats which had received the thromboxane A_2 antagonist ICI 192,605 either alone or in combination with ICI 170,809 in both studies (Figure 2c). Similarly, the responses to the combination of ADP, 5-HT and U46619 were attenuated by the thromboxane A_2 antagonist ICI 192,605 at either dose, given alone or when given with the 5-HT$_2$ antagonist ICI 170,809 (Figure 2d).

Effects of ICI 170,809 and ICI 192,605 on collagen-induced platelet aggregation

In these experiments when collagen 1 μg ml^{-1} was used as the primary aggregating agent, either 5-HT 10 μM or U46619 1 μM caused a similar degree of potentiation of the response to collagen and the largest responses were seen when both 5-HT and U46619 were added before collagen (Figure 3). No differences were observed in the response to collagen alone, between the groups which received both vehicles, ICI 170,809, ICI 192,605 or both drugs in either the low or high dose studies (Figure 3a).

In the high dose study however, the ability of 5-HT to potentiate the collagen response was reduced in blood taken from rats which had been pretreated with the 5-HT$_2$ antagonist ICI 170,809, whether given alone or in combination with ICI 192,605, whereas the effect of 5-HT was preserved in blood taken from rats pretreated with the thromboxane A_2 antagonist alone (Figure 3b). Similarly, the U46619-induced enhancement of the response to collagen was only prevented by the thromboxane A_2 antagonist ICI 192,605 (Figure 3c). When both 5-HT and U46619 were used to enhance the response to collagen, it was only in blood taken from rats which had received both antagonists that the response was significantly reduced when compared to that in controls (Figure 3d).

A different pattern was observed in the low dose study. Neither ICI 170,809 0.3 mg kg^{-1}, nor ICI 192,605 0.3 mg kg^{-1} min^{-1}, given alone or in combination significantly altered the responses to collagen plus 5-HT (Figure 3b), or collagen plus U46619 (Figure 3c). It was only in blood taken from rats that had received both antagonists that the response to combined addition of 5-HT, U46619 and collagen was sig-

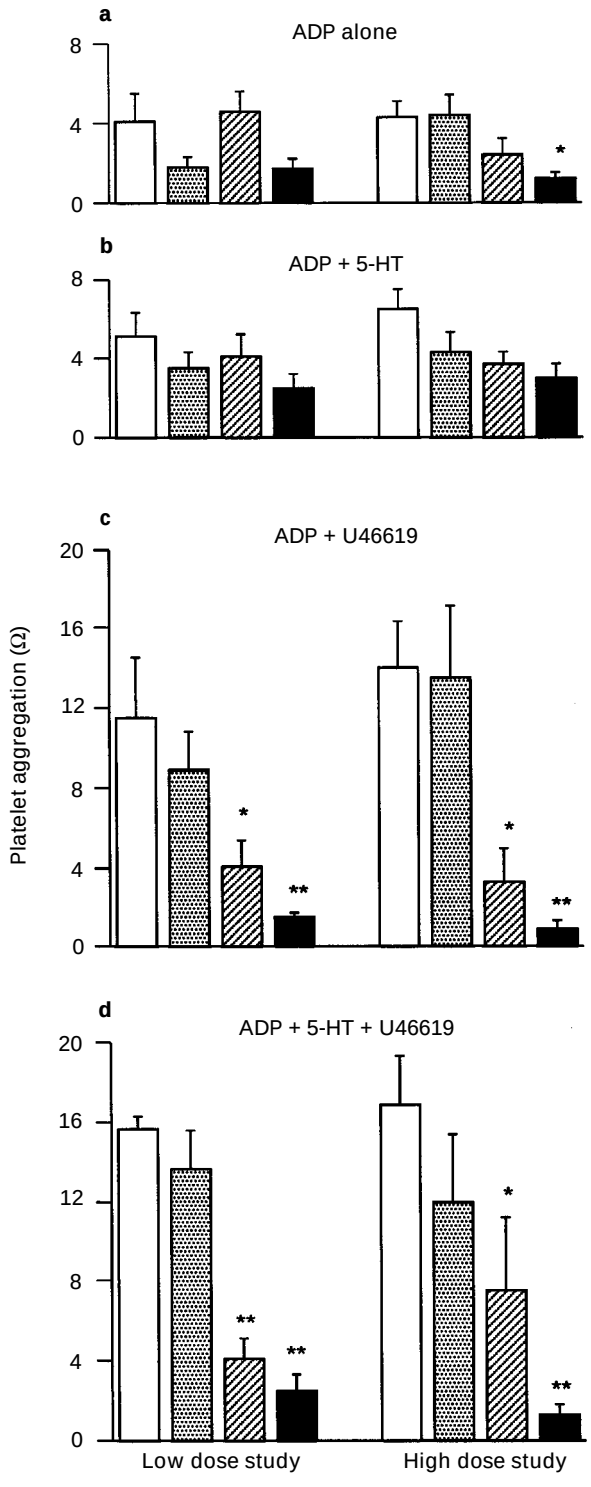

Figure 2 The effects of pretreatment of anaesthetized rats with vehicles (control, open columns), the 5-HT$_2$ antagonist ICI 170,809 (stippled columns), the thromboxane A$_2$ antagonist ICI 192,605 (hatched columns), or both drugs (solid columns) on ADP-induced platelet aggregation measured *ex vivo* in whole blood. (a) The effects of ADP 0.1 μM alone; (b) 5-HT 10 μM added 15 s before ADP 0.1 μM; (c) U46619 1 μM added 1 min before ADP 0.1 μM; and (d) U46619 1 μM and 5-HT 10 μM added before ADP 0.1 μM. In the low dose study (left hand columns) the doses were, ICI 170,809 0.3 mg kg^{-1} and ICI 192,605 0.3 mg kg^{-1} min^{-1}; in the high dose study (right hand columns) the doses were 1 mg kg^{-1} and 1 mg kg^{-1} min^{-1}, respectively. Each value is the mean$\pm$s.e.mean, $n=6$. *$P<0.05$, **$P<0.01$ compared to corresponding value in the control group, Kruskal Wallis test.

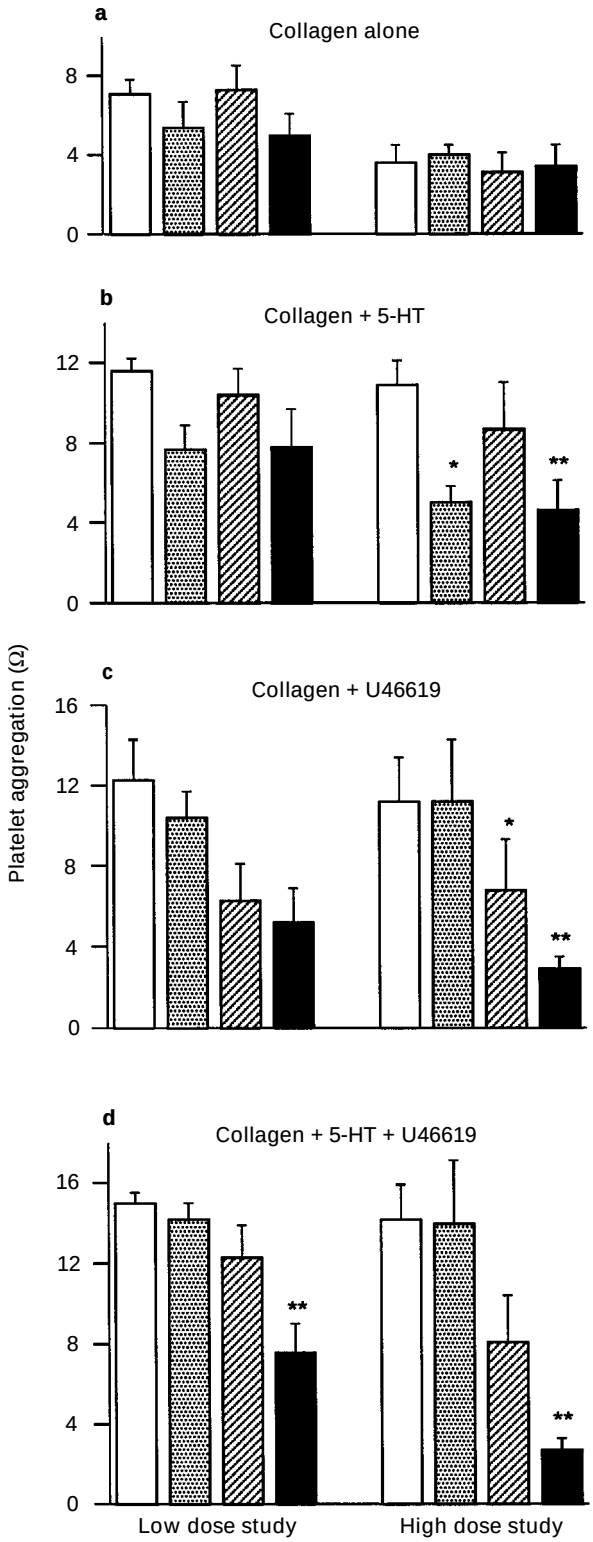

Figure 3 The effects of pretreatment of anaesthetized rats with vehicles (control, open columns), the 5-HT$_2$ antagonist ICI 170,809 (stippled columns), the thromboxane A$_2$ antagonist ICI 192,605 (hatched columns), or both drugs (solid columns) on collagen-induced platelet aggregation measured *ex vivo* in whole blood. (a) The effects of collagen 1 μg ml^{-1} alone; (b) 5-HT 10 μM added 15 s before collagen 1 μg ml^{-1}; (c) U46619 1 μM added 1 min before collagen 1 μg ml^{-1}; and (d) U46619 1 μM and 5-HT 10 μM added before collagen 1 μg ml^{-1}. In the low dose study (left hand columns) the doses were, ICI 170,809 0.3 mg kg^{-1} and ICI 192,605 0.3 mg kg^{-1} min^{-1}; in the high dose study (right hand columns) the doses were 1 mg kg^{-1} min^{-1} respectively. Each value is the mean$\pm$s.e.mean, $n=6$. *$P<0.05$, **$P<0.01$ compared to corresponding value in the control group, Kruskal Wallis test.

nificantly less than in blood taken from control rats (Figure 3d). Thus the lower doses of the antagonists had relatively little ability to attenuate the ability of 5-HT or U46619 to amplify platelet aggregation to collagen. However, when rats were pretreated with the higher doses of the antagonists, each antagonist suppressed the amplifying effects of the corresponding agonist and marked suppression of collagen-induced platelet aggregation occurred when both antagonists were given together.

Effects of ICI 170,809 and ICI 192,605 on platelet counts and haemodynamics

In the high dose study, platelet counts in whole blood taken from anaesthetized rats which had received either vehicles (controls), ICI 170,809, ICI 192,605 or both drugs did not differ significantly. The values were 1021 ± 38, 952 ± 39, 1004 ± 76 and 929 ± 49 ($\times 10^9$) platelets l^{-1} in each group, respectively. The effects of ICI 170,809, ICI 192,605 and their combined administration on heart rate and arterial blood pressure are detailed in Table 1. Neither drug alone significantly altered heart rate, systolic or diastolic blood pressure. However, combined administration of ICI 170,809 1 mg kg^{-1} and ICI 192,605 1 mg kg^{-1} min^{-1} reduced both systolic and diastolic blood pressure (Table 1).

Effects of ICI 170,809 and ICI 192,605 on isolated cardiac muscle

The 5-HT$_2$ antagonist ICI 170,809 increased the effective refractory period in isolated left papillary muscles (Figure 4) and right ventricular strips (Figure 5). These effects reached statistical significance 10 min after addition of 30 μM ICI 170,809 in both preparations. In contrast, the thromboxane A$_2$ antagonist ICI 192,605 did not increase effective refractory period at all, even after 15 min in the presence of 100 μM (Figures 4 and 5). When a separate group of tissues was exposed to increasing concentrations of ICI 170,809 in the presence of the highest concentration of ICI 192,605 (100 μM), the effects of ICI 170,809 on effective refractory period were not altered in either left papillary muscles (Figure 4) or right ventricular strips (Figure 5).

The effects of concentrations of ICI 170,809 above 30 μM could not be measured because of contractile failure. Developed tension was relatively well maintained during the course of these experiments, but in each preparation, after addition of either 30 or 100 μM ICI 170,809, contractile failure occurred. This can be seen in Table 2 for left papillary muscles and Table 3 for right ventricular strips, where the number of surviving tissues is given along with the values for developed tension. ICI 192,605 had no effect on developed tension and did not alter the effect of ICI 170,809 on developed tension (Tables 2 and 3).

Table 1 Heart rate, systolic and diastolic blood pressure (BP) measured 1 min before and 10 min after drug or vehicle administration in anaesthetized rats in the high dose study on platelet aggregation

	Pre	*Post*
Heart rate (beats min^{-1})		
Control	355 ± 10	331 ± 14
ICI 170,809 (1 mg kg^{-1})	382 ± 10	328 ± 14
ICI 192,605 (1 mg kg^{-1} min^{-1})	347 ± 24	312 ± 25
Both drugs	335 ± 9	305 ± 14
Systolic BP (mmHg)		
Control	128 ± 9	129 ± 7
ICI 170,809 (1 mg kg^{-1})	133 ± 10	123 ± 9
ICI 192,605 (1 mg kg^{-1} min^{-1})	120 ± 13	120 ± 11
Both drugs	128 ± 10	100 ± 2*
Diastolic BP (mmHg)		
Control	105 ± 6	109 ± 6
ICI 170,809 (1 mg kg^{-1})	110 ± 9	103 ± 11
ICI 192,605 (1 mg kg^{-1} min^{-1})	98 ± 10	95 ± 9
Both drugs	103 ± 7	82 ± 3*

Values are the mean $\pm$ s.e.mean of $n = 6$. *$P < 0.05$ compared to corresponding value in the control group, one way analysis of variance and modified t test (Bonferroni correction).

Discussion

The main aim of the current studies was to try to elucidate possible mechanisms that could explain the marked antiarrhythmic activity of combined administration of the 5-HT$_2$ antagonist ICI 170,809 and the thromboxane A$_2$ antagonist ICI 192,605. Since drugs that have any type of 'membrane

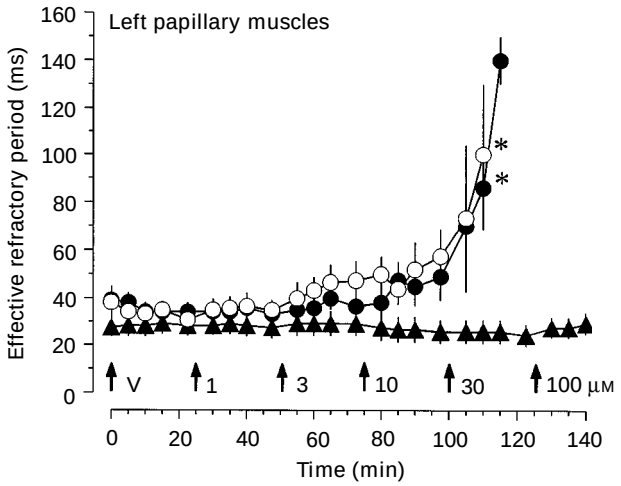

Figure 4 The effects of vehicle (V), and increasing concentrations of the 5-HT$_2$ antagonist ICI 170,809 (●), the thromboxane A$_2$ antagonist ICI 192,605 (▲), and the combination of increasing concentrations of ICI 170,809 in the presence of a fixed concentration of 100 μM ICI 192,605 (○) on the effective refractory period in rat isolated left papillary muscles. Each value is the mean and vertical lines show s.e.mean, $n = 6$. *$P < 0.05$ compared to value at time 0 min within group and compared to the value in the ICI 192,605 group at the same time point, Kruskal Wallis test.

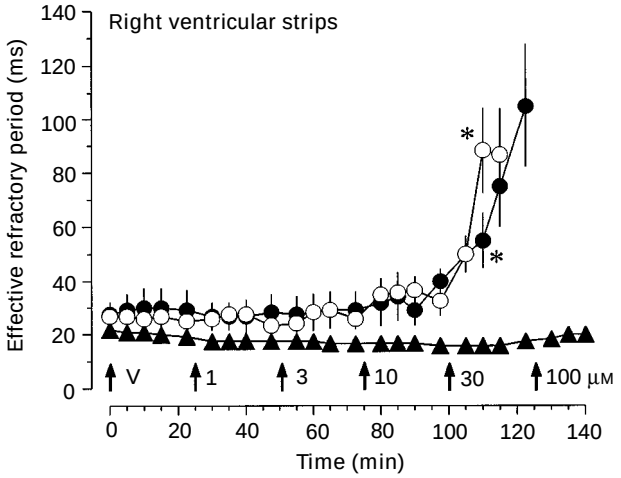

Figure 5 The effects of vehicle (V) and increasing concentrations of the 5-HT$_2$ antagonist ICI 170,809 (●), the thromboxane A$_2$ antagonist ICI 192,605 (▲), and the combination of increasing concentrations of ICI 170,809 in the presence of a fixed concentration of 100 μM ICI 192,605 (○) on the effective refractory period in rat isolated right ventricular strips. Each value is the mean and vertical lines show s.e.mean, $n = 6$. *$P < 0.05$ compared to value at time 0 min within group and compared to the value in the ICI 192,605 group at the same time point, Kruskal Wallis test.

stabilizing' activity could theoretically be antiarrhythmic, the direct effects of these compounds on rat isolated ventricular preparations were investigated. Only the 5-HT$_2$ antagonist ICI 170,809 had any effect on these isolated cardiac muscle preparations. Both the effective refractory period and the contractile function of the tissues were affected, although in a different manner. Whilst the effective refractory period increased gradually with time and as the concentrations of the drug increased, the contractile function was well maintained until a certain point was reached when an abrupt decline occurred. Significant increases in the effective refractory period were detected before contractile failure occurred. It is possible that the decline in contraction was a consequence of the effective refractory period being lengthened to such an extent that the tissues became inexcitable. The concentration of ICI 170,809 required to prolong the effective refractory period significantly was similar to that which had been shown previously to decrease the maximum driving frequency of rat atrial and ventricular preparations (Ellis & Coker, 1992).

Table 2 Developed tension in rat isolated left papillary muscles in the presence of ICI 170,809, ICI 192,605 or ICI 170,809 in the presence of a fixed concentration of ICI 192,605 (100 μM)

	Developed tension (mN)			
	0 min	*5 min*	*10 min*	*15 min*
ICI 170,809 alone				
Vehicle	2.9±0.6	2.8±0.6	2.7±0.5	2.6±0.5
1 μM	2.7±0.6	2.6±0.6	2.5±0.6	2.6±0.6
3 μM	2.5±0.5	2.4±0.6	2.3±0.7	2.3±0.6
10 μM	2.5±0.6	2.6±0.7	2.6±0.7	2.5±0.7
30 μM	2.9±0.7	2.6±0.6	2.3±0.7	2.3±0.9
(*n*)	(5)	(5)	(5)	(4)
100 μM	~	~	~	~
ICI 192,605 alone				
Vehicle	2.3±0.4	2.4±0.4	2.3±0.4	2.2±0.4
1 μM	2.4±0.4	2.3±0.4	2.1±0.4	2.1±0.4
3 μM	2.3±0.3	2.2±0.4	2.1±0.4	2.1±0.4
10 μM	2.5±0.5	2.0±0.3	1.9±0.3	1.9±0.3
30 μM	2.2±0.4	2.0±0.3	1.9±0.3	1.9±0.3
100 μM	2.4±0.5	2.3±0.4	2.2±0.4	2.2±0.4
ICI 170,809+ICI 192,605 100 μM				
Vehicle	2.8±0.3	2.3±0.3	2.1±0.3	1.9±0.3
1 μM	1.9±0.3	1.8±0.2	1.6±0.2	1.5±0.2
3 μM	1.7±0.3	1.5±0.3	1.5±0.3	1.4±0.3
10 μM	1.5±0.3	1.4±0.3	1.2±0.2	1.2±0.3
30 μM	1.6±0.4	1.5±0.3	1.2	1.0
(*n*)	(5)	(4)	(2)	(2)
100 μM	~	~	~	~

Each value is the mean±s.e.mean of $n=6$ unless stated otherwise. ~ no surviving tissues at this time point.

Table 3 Developed tension in rat isolated right ventricular strips in the presence of ICI 170,809, ICI 192,605 or ICI 170,809 in the presence of a fixed concentration of ICI 192,605 (100 μM)

	Developed tension (mN)			
	0 min	*5 min*	*10 min*	*15 min*
ICI 170,809 alone				
Vehicle	3.8±1.0	3.8±1.0	3.6±1.0	3.6±1.0
1 μM	3.8±1.0	3.6±1.0	3.5±0.9	3.4±0.9
3 μM	3.6±1.0	3.4±0.9	3.4±0.9	3.3±0.9
10 μM	3.5±1.1	2.8±1.1	3.2±1.0	2.9±0.9
30 μM	3.6±0.9	3.0±0.7	2.6±0.5	2.4±0.5
(*n*)	(5)	(5)	(4)	(4)
100 μM	2.2±0.6	0.7±0.1	~	~
(*n*)	(4)	(3)		
ICI 192,605 alone				
Vehicle	4.0±0.9	4.0±0.8	3.9±0.8	3.8±0.8
1 μM	3.7±0.9	3.9±0.8	3.7±0.8	3.6±0.7
3 μM	3.5±0.8	3.6±0.7	3.3±0.7	3.2±0.7
10 μM	3.2±0.8	3.4±0.7	3.3±0.7	3.2±0.7
30 μM	3.4±0.8	3.1±0.8	3.1±0.9	2.9±0.8
100 μM	3.5±0.7	3.4±0.8	3.3±0.7	3.0±0.7
ICI 170,809+ICI 192,605 100 μM				
Vehicle	4.8±1.3	4.2±1.3	3.9±1.3	3.6±1.2
1 μM	3.7±1.1	3.4±0.9	3.1±0.9	3.0±0.8
3 μM	3.4±0.9	3.2±0.9	3.0±0.8	2.9±0.7
10 μM	3.2±0.8	3.0±0.8	2.7±0.7	2.8±0.7
30 μM	2.8±0.7	2.4±0.8	2.0±0.6	2.4±0.6
(*n*)	(6)	(6)	(6)	(4)
100 μM	2.6	~	~	~
(*n*)	(2)			

Each value is the mean±s.e.mean of $n=6$ unless stated otherwise. ~ no surviving tissues at this time point.

Whether or not these effects of ICI 170,809 on isolated cardiac muscle are a consequence of blockade of 5-HT$_2$ receptors cannot be determined from the present study. However, it seems unlikely that this is the case, since the concentration at which the increase in the effective refractory period became significant (30 μM) is far in excess of that required to block 5-HT$_2$ receptors (Frenken & Kaumann, 1989). The lack of prolongation of the effective refractory period by the thromboxane A$_2$ antagonist ICI 192,605 and its inability to enhance the effect of ICI 170,809 indicate that direct effects on cardiac muscle are not responsible for the *in vivo* antiarrhythmic activity observed previously with combined administration of these drugs.

These findings reinforce the argument that the lack of suppression of ischaemia-induced arrhythmias by combined administration of ICI 170,809 and ICI 192,605 (Shaw & Coker, 1996a) suggests that these drugs do not act directly on the cardiac myocytes. If a drug or combination directly altered the electrical stability of cardiac myocytes it would be expected to reduce arrhythmias irrespective of whether they were induced by ischaemia or reperfusion. It has been suggested that the factors precipitating ischaemia-induced and reperfusion-induced arrhythmias differ. Reperfusion-induced arrhythmias are resistant to some standard antiarrhythmic drugs (Naito *et al*., 1981) and it has been argued that they may be a consequence of the abrupt washout of certain products that have accumulated during ischaemia (Corbalan *et al*., 1976). These products could include platelets which have aggregated as a result of the rapid increase in thromboxane A$_2$ in blood draining from the ischaemic region (Coker *et al*., 1981; Coker, 1984). Thus the other main aim of these studies was to investigate platelet responsiveness to 5-HT and the thromboxane A$_2$ mimetic U46619 and how this might be altered by ICI 170,809 and ICI 192,605.

The results of the current platelet studies demonstrate that both 5-HT and U46619 can amplify platelet aggregation in rat whole blood. When collagen was used as the primary aggregating agent both 5-HT and U46619 caused a similar degree of potentiation of the collagen response, although U46619 was about 10 times more potent. However, a different pattern was observed when ADP was the primary aggregating agent. In the preliminary studies, addition of 5-HT alone did significantly potentiate the response to a minimally effective concentration of ADP, but not to the same extent as U46619. In contrast, in blood from the controls for both the low and high dose antagonist studies, although 5-HT appeared to potentiate the response to ADP, this apparent difference was not statistically significant.

It should also be noted that in the preliminary studies when 5-HT and U46619 were both added to platelets, substantial aggregation occurred in blood from 4 out of 6 rats, whereas in blood from the control rats for the drug studies this effect was not seen. The controls for the drug studies received both vehicles, a bolus of acidified water and an infusion of alkaline saline. Perhaps the failure of 5-HT to amplify significantly responses to either U46619 or ADP could be due to an effect of the vehicles. Alternatively, the longer time interval between

completion of the surgical preparation and the withdrawal of blood in the drug studies compared with the preliminary study may account for the difference. A longer interval would allow more time for post-operative elevations in circulating catecholamine concentrations to decline. Adrenaline is known to be capable of potentiating 5-HT-induced platelet aggregation (De Clerck *et al*., 1988; Belcher *et al*., 1992; McAuliffe *et al*., 1993).

To mimic the previous experiments on arrhythmias as closely as possible, platelet aggregation was measured *ex vivo* in whole blood after administration of ICI 170,809 and ICI 192,605 to anaesthetized rats. The lack of significant potentiation of ADP-induced platelet aggregation by 5-HT in the drug studies makes it difficult to draw firm conclusions about the possible activity of the antagonists. However, with collagen as the primary aggregating agent, a clear pattern emerges. The higher doses of each antagonist blocked the effects of the corresponding agonist and marked suppression of platelet aggregation was observed with combined administration of both antagonists. In contrast, the lower doses of the antagonists were less effective in preventing enhancement of collagen-induced platelet aggregation by 5-HT and U46619. This pattern corresponds to that seen in our previous studies on reperfusion-induced arrhythmias (Shaw & Coker, 1996a,b), where only the combination of the higher doses of the antagonists significantly reduced ventricular tachycardia and ventricular fibrillation.

In some species 5-HT is a weak aggregating agent (De Clerck & Herman, 1983), but in rat citrated platelet rich plasma it does not cause aggregation, although it can amplify responses to other agents (Ellis & Coker, 1992). The present results confirm that the same is true in rat whole blood. Thromboxane A_2 is a strong aggregating agent producing substantial responses in some species e.g. human and rabbit platelets (Takahara *et al*., 1990; Shaw, 1994). However, in dog platelets thromboxane A_2 alone does not cause aggregation (Chignard & Vargaftig, 1976) but small elevations in adrenaline concentrations will reveal a response to thromboxane A_2 (Johnson *et al*., 1980). The current studies indicate that the response to thromboxane A_2 in rat platelets is similar, since the thromboxane A_2 mimetic U46619 did not produce any aggregation on its own but potentiated responses to ADP and collagen.

The concentrations of ADP and collagen used in these studies were the minimum concentrations which always gave a measurable response and were chosen from the preliminary studies. Since 5-HT and U46619 significantly increased responses to collagen, despite having no effect themselves, this indicates the occurrence of a synergistic interaction. However, the requirement for blockade of both 5-HT$_2$ and TP receptors to cause marked reductions in platelet aggregation, suggests that the interaction between endogenous 5-HT and thromboxane A_2 displays redundancy. Either agonist can compensate for the loss or inactivity of the other, thus explaining why both antagonists are necessary. To explore these relationships further, full concentration-response curves would need to be examined (Leff, 1987), this was not feasible in the present studies because of the limited volume of blood available from each rat.

Enhanced antiplatelet or antithrombotic activity has been shown with combinations of other 5-HT$_2$ and TP receptor blockers. A brief report has suggested that the 5-HT$_2$ antagonist ketanserin and the thromboxane A_2 antagonist BM 13.177 given together had greater antiplatelet activity than either drug alone (De Clerck *et al*., 1986). Combinations of LY53857 and SQ29548 (Golino *et al*., 1989) or ketanserin and ridogrel (Vandeplassche *et al*., 1993) enhanced thrombolysis in canine models. It is possible that some of the beneficial effects of combinations of 5-HT$_2$ and thromboxane A_2 antagonists could be a consequence of reduced coronary vasospasm (Bax *et al*., 1994). However, there is evidence that at least part of the contractile response of coronary arteries to 5-HT is due to stimulation of 5-HT$_{1D}$ receptors and that this can contribute to synergistic interactions with thromboxane A_2 (Chester *et al*., 1993; Cocks *et al*., 1993). Previously, it was concluded that the antiarrhythmic effect of combined administration of ICI 170,809 and ICI 192,605 was unlikely to be due to vascular effects of the drugs (Shaw & Coker, 1996a). Although significant differences in blood pressure were only seen in the rats that received both drugs in the present study, the pattern of haemodynamic effects was similar to that observed previously, where the effects of the lower doses of the drugs were similar to those of the higher doses (Shaw & Coker, 1996a, 1997). It should also be noted that there were only 6 rats per group in the present study whereas 12 per group were required for the arrhythmia studies. These observations suggest that the former conclusion is still valid.

There is a growing body of evidence indicating that intracoronary platelet aggregation can cause arrhythmias, and the contribution of platelet activation during myocardial ischaemia to arrhythmogenesis has been reviewed recently (Flores, 1996). One important point made in this review is that the adhesion of platelets to collagen that is exposed when there is endothelial damage is likely to be the critical event initiating thrombosis *in vivo*. Occluding a coronary artery by tying a ligature around it is likely to damage the full thickness of the arterial wall including the endothelium. Initiating reperfusion by rapid release of the ligature, as was done in the arrhythmia experiments (Shaw & Coker, 1996a), is likely to dislodge adherent platelet thrombi, which will be flushed downstream where they may lodge and precipitate events leading to severe arrhythmias such as ventricular fibrillation. Thus interventions which prevent or reduce the effects of collagen on platelet adhesion and aggregation may be particularly beneficial in these circumstances. The relative effectiveness of ICI 170,809 and ICI 192,605 against collagen-induced platelet aggregation closely resembles their relative effectiveness against reperfusion-induced arrhythmias.

In conclusion therefore, these studies show that combined administration of the 5-HT$_2$ antagonist ICI 170,809 and the thromboxane A_2 antagonist ICI 192,605 caused greater inhibition of platelet aggregation than either drug alone, whereas only ICI 170,809 altered the effective refractory period of cardiac muscle. This indicates that the significant antiarrhythmic activity that was observed previously (Shaw & Coker, 1996a) when ICI 170,809 and ICI 192,605 were given in combination is much more likely to be due to reduced platelet aggregation than to direct effects on cardiac muscle.

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References

ASHTON, J.H., OGLETREE, M.L., MICHEL, I.M., GOLINO, P. MCNATT, J.M., TAYLOR, A.L., RAHEJA, A., SCHMITZ, J., BUJA, L.M. & CAMPBELL, W.B. (1987).Co-operative mediation by serotonin S_2 and thromboxane A_2/prostaglandin H_2 receptor activation of cyclic flow variations in dogs with severe coronary artery stenoses. *Circulation*, **76,** 952–959.

BARNES, C.S. & COKER, S.J. (1995). Failure of nitric oxide donors to alter arrhythmias induced by acute myocardial ischaemia in anaesthetized rats. *Br. J. Pharmacol.*, **114,** 349–356.

BAX, W.A., RENZENBRINK, G.J., VAN DER LINDEN, E.A., ZIJLSTRA, F.J., VAN HEUVEN-NOLSEN, D., FEKKES, D., BOS, E. & SAXENA, P.R. (1994). Low-dose aspirin inhibits platelet-induced contraction of the human isolated coronary artery. A role for additional 5-hydroxytryptamine receptor antagonism against coronary vasospasm? *Circulation,* **89,** 623–629.

BELCHER, P.R., DRAKE-HOLLAND, A.J., HYND, J. & NOBLE, M.I.M. (1992). Dispersion of coronary artery thrombi by antagonism of platelet serotonin receptor in the dog. *Cardiovasc. Res.*, **26,** 292–296.

BLACKBURN, T.P., THORNBER, C.W., PEARCE, R.J. & COX, B. (1988). *In vitro* pharmacology of ICI 170,809–a new 5-HT$_2$ antagonist. *FASEB. J.*, **2,** A1404.

CARDINAL, D.C. & FLOWER, R.J. (1980). The electronic aggregometer: a novel device for assessing platelet behaviour in blood. *J. Pharmacol. Methods*, **3,** 135–158.

CHESTER, A.H., ALLEN, S.P., TADJKARIMI, S. & YACOUB, M.H. (1993). Interaction between thromboxane A$_2$ and 5-hydroxytryptamine receptor subtypes in human coronary arteries. *Circulation*, **87,** 874–880.

CHIGNARD, M. & VARGAFTIG, B.B. (1976). Dog platelets fail to aggregate when they form aggregating substances upon stimulation with arachidonic acid. *Eur. J. Pharmacol.*, **38,** 7–18.

COCKS, T.M., KEMP, B.K., PRUNEAU, D. & ANGUS, J.A. (1993). Comparison of contractile responses to 5-hydroxytryptamine and sumatriptan in human isolated coronary: synergy with the thromboxane A$_2$-receptor agonist, U46619. *Br. J. Pharmacol.*, **110,** 360–368.

COKER, S.J. (1984). Further evidence that thromboxane exacerbates arrhythmias: effects of UK38485 during coronary artery occlusion and reperfusion in anaesthetized greyhounds. *J. Mol. Cell. Cardiol.*, **16,** 633–641.

COKER, S.J. & BATEY, A.J. (1995). Effective refractory period and contractility of isolated cardiac muscle in the presence and absence of 5-HT$_2$ and thromboxane antagonists alone and in combination. *Br. J. Pharmacol.*, **116,** 284P.

COKER, S.J., PARRATT, J.R., LEDINGHAM, I.McA. & ZEITLIN, I.J. (1981). Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. *Nature*, **291,** 343–344.

CORBALAN, R., VERRIER, R.L. & LOWN, B. (1976). Differing mechanisms for ventricular vulnerability during coronary artery occlusion and release. *Am. Heart J.*, **92,** 223–230.

DE CLERCK, F.F. & HERMAN, A.G. (1983). 5-Hydroxytryptamine and platelet aggregation. *Fedn. Proc.*, **42,** 228–232.

DE CLERCK, F.F., XHONNEUX, B., VAN GORP, L., BEETENS, J. & JANSSEN, P.A.J. (1986). S$_2$-serotonergic receptor inhibition (ketanserin), combined with thromboxane A$_2$/prostaglandin endoperoxide receptor blockade (BM 13.177): enhanced antiplatelet effect. *Thromb. Haemostas.*, **56,** 236.

DE CLERCK, F.F., XHONNEUX, B. & DE CHAFFOY DE COURCELLES, D. (1988). Functional expression of the amplification reaction between serotonin and epinephrine on platelets. *J. Cardiovasc. Pharmacol.*, **11** (Suppl. 1), S1–S5.

ELLIS, A.M. & COKER, S.J. (1992). Contribution of antiplatelet activity to the effects of 5-HT$_2$ receptor antagonists on reperfusion-induced arrhythmias in anaesthetized rats. *Eur. J. Pharmacol.*, **219,** 97–104.

FLORES, N.A. (1996). Platelet activation during myocardial ischaemia: a contributory arrhythmogenic mechanism. *Pharmacol. Ther.*, **72,** 83–108.

FRENKEN, M. & KAUMANN, A.J. (1989). Dimethylation of the activator ICI 169,369 results in a high affinity partial deactivator, ICI 170,809, of the arterial 5-hydroxytryptamine$_2$ receptor system. *J. Pharmacol. Exp. Ther.*, **250,** 707–713.

GOLINO, P., ASHTON, J.H., MCNATT, J., GLAS-GREENWALT, P., SHENG-KUN, Y., O'BRIEN, R.A., BUJA, L.M. & WILLERSON, J.T. (1989). Simultaneous administration of thromboxane A$_2$- and serotonin S$_2$-receptor antagonists markedly enhances thrombolysis and prevents or delays reocclusion after tissue-type plasminogen activator in a canine model of coronary thrombosis. *Circulation*, **79,** 911–919.

JESSUP, C.L., JESSUP, R. & WAYNE, M. (1988). The effects of ICI 192,605, a selective thromboxane A$_2$ receptor antagonist, on platelets. *Br. J. Pharmacol.*, **33,** 676P.

JOHNSON, G.J., RAO, G.H.R., LEIS, L.A. & WHITE, J.G. (1980). Effects of agents that alter cyclic-AMP on arachidonate-induced platelet aggregation in the dog. *Blood*, **55,** 722–729.

LEFF, P. (1987). An analysis of amplifying and potentiating interactions between agonists. *J. Pharmacol. Exp. Ther.*, **243,** 1035–1042.

MCAULIFFE, S.J.G., SNOW, H.M., COX, B., SMITH, C.C.T. & NOBLE, M.I.M. (1993). Interaction between the effects of 5-hydroxytryptamine and adrenaline on the growth of platelet thrombi in the coronary artery of the anaesthetized dog. *Br. J. Pharmacol.*, **109,** 405–410.

MCAULIFFE, S.J.G., MOORS, J.A. & JONES, H.B. (1994). Comparative effects of anti-platelet agents as adjuncts to tissue plasminogen activator in a dog model of occlusive coronary thrombosis. *Br. J. Pharmacol.*, **112,** 272–276.

MILLSON, D.S., JESSUP, C.J., SWAISLAND, A., HAWORTH, S., RUSHTON, A. & HARRY, J.D. (1992). The effects of a selective 5-HT$_2$ receptor antagonist (ICI 170,809) on platelet aggregation and pupillary responses in healthy volunteers. *Br. J. Clin. Pharmacol.*, **33,** 281–288.

NAITO, M., MICHELSON, R.L., KMETZO, J.J., KAPLINSKY, E. & DREIFUS, L.S. (1981). Failure of antiarrhythmic drugs to prevent experimental reperfusion ventricular fibrillation. *Circulation*, **63,** 70–79.

SCHOLTYSIK, G. (1980). Retardation of aconitine-induced ECG-alterations in rats as an indication of membrane-stabilizing drug effects. In *The Rat Electrocardiogram in Pharmacology and Toxicology*, ed. Budden, R., Detweiler, D.K. & Zbinden, G. pp. 257–264. Oxford: Pergamon Press Ltd.

SHAW, L.A. (1994). The influence of platelet derived factors and cholesterol on arrhythmogenesis. *PhD Thesis, The University of Liverpool.*

SHAW, L.A. & COKER, S.J. (1994). Profound reductions in platelet responses after combined administration of 5-HT$_2$ and thromboxane antagonists. *Br. J. Pharmacol.*, **112,** 479P.

SHAW, L.A. & COKER, S.J. (1996a). Suppression of reperfusion-induced arrhythmias with combined administration of 5-HT$_2$ and thromboxane A$_2$ antagonists. *Br. J. Pharmacol.*, **117,** 817–822.

SHAW, L.A. & COKER, S.J. (1996b). Erratum (*Br. J. Pharmacol.*, **117,** 817–822.) *Br. J. Pharmacol.*, **118,** 1326.

SHAW, L.A. & COKER, S.J. (1997). Erratum (*Br. J. Pharmacol.*, **117,** 817–822.) *Br. J. Pharmacol.*, **120,** 1186.

SIESS, W. (1989). Molecular mechanisms of platelet activation. *Physiol. Rev.*, **69,** 58–178.

STEEN, V.M. & HOLMSEN, H. (1987). Current aspects on human platelet activation and responses. *Eur. J. Haematol.*, **38,** 383–399.

TAKAHARA, K., MURRAY, R., FITZGERALD, G.A. & FITZGERALD, D.J. (1990). The response to thromboxane A$_2$ analogues in human platelets. *J. Biol. Chem.*, **265,** 6836–6844.

VANDEPLASSCHE, G., HERMANS, C., VAN DAEL, L., WOUTERS, L. & DE CLERCK, F. (1993). Interplay between platelet derived 5-HT and arachidonic acid metabolites limits the thrombolytic efficacy of streptokinase against canine platelet rich coronary thrombosis. *J. Cardiovasc. Pharmacol.*, **21,** 56–69.

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