Interaction between the effects of 5-hydroxytryptamine and adrenaline on the growth of platelet thrombi in the coronary artery of the anaesthetized dog

*S.J.G. McAuliffe, *¹H.M. Snow, *B. Cox, †C.C.T. Smith & ‡M.I.M. Noble

*Bioscience II, ICI Pharmaceuticals, Macclesfield; †Department of Clinical Pharmacology, University College and Middlesex Medical School and ‡Academic Unit of Cardiovascular Medicine, Charing Cross and Westminster Medical School, London

- 1 The interaction between adrenaline and 5-hydroxytryptamine (5-HT) has been quantitated on the rate of thrombus formation, in the stenosed coronary artery with damaged endothelium of the anaesthetized dog.
- 2 Changes in the plasma concentration of adrenaline were produced by varying the rate of an intravenous infusion of adrenaline and in the effects of 5-HT, by intravenous injections of the selective 5-HT₂ receptor antagonist, ICI 170809.
- 3 Increases in the plasma concentration of adrenaline, which did not cause significant changes in blood pressure and heart rate, increased the rate of thrombus formation.
- 4 Antagonism of the 5-HT₂ receptor by ICI 170809, in the absence of an infusion of adrenaline, abolished thrombus formation (mean ED₅₀ 0.41 µg kg⁻¹, i.v.).
- 5 The effects of adrenaline were non-competitively antagonized by ICI 170809; maximum effects were obtained in the dose-range 50–200 µg kg⁻¹, i.v., when the mean dose-ratio increase in adrenaline required to restore equivalent rates of thrombus formation was 39 fold.
- 6 These results are consistent with a synergism between adrenaline and 5-HT and emphasize the importance of both on thrombus formation.

Keywords: Platelet; coronary artery; 5-hydroxytryptamine; adrenaline

Introduction

Platelet thrombi form at sites of arterial stenosis and endothelial denudation or damage. The presence of such sites in the coronary arteries of patients dying suddenly has led to the suggestion that the acute formation of platelet thrombi plays an important role in coronary artery disease (Haerem, 1972).

Folts and co-workers (1976, 1982) developed a model of thrombus formation in the stenosed coronary artery of the dog with damaged endothelium. In this model, coronary blood flow declines as a thrombus forms, until occlusion or near occlusion occurs, when the platelet thrombus dislodges and flow is restored. Thus a cyclical pattern of flow reductions is established (CFRs). This pattern of CFRs has now been observed in human coronary arteries (Eichorn *et al.*, 1991). The arterial thrombi contain platelets, fibrin and red and white blood cells. Arterial thrombus formation appears to be initiated by activation of platelets and the subsequent release of 5-hydroxytryptamine (5-HT) and thromboxane A₂, both of which promote further aggregation (Ashton *et al.*, 1986; De Clerck *et al.*, 1988). This positive feedback promotion of thrombus formation can be potentiated by adrenaline (Ashton *et al.*, 1989).

The aim of the present experiments, in anaesthetized dogs, was to quantify the interaction between the effects of adrenaline and 5-HT on thrombus formation, in a stenosed and damaged coronary artery, using the rate of decline of coronary blood flow as an index of the rate of thrombus formation. A preliminary communication of the results has been given (Cox *et al.*, 1991a,b).

Methods

General

Experiments were carried out in 19 beagle dogs weighing between 15.0–19.0 kg. The dogs were sedated with morphine sulphate (10 mg, i.m.) and 30 min later were anaesthetized with pentobarbitone (induction 30 mg kg⁻¹, i.v., maintenance 3 mg kg⁻¹, i.v., every 30 min) through a cannula inserted into the long saphenous vein under local anaesthesia.

Ventilation was delivered via a tracheotomy with a mixture of 40% oxygen in room air supplied by a Harvard animal ventilator. When the chest was open a resistance to expiration was produced by placing the expiratory output of the pump under 3 cm of water. Samples of arterial blood were withdrawn at intervals throughout each experiment and pH, P_{O2} and P_{CO2} measured with a Corning EEL blood gas analyser. End-tidal P_{CO2} was continuously measured by aspirating expired air from the trachea into an infra-red CO₂ analyser (P.K. Morgan Ltd.). The arterial P_{CO2} was kept within the limits 34–42 mmHg and the arterial blood pH between 7.33 and 7.43 either by adjustment of respiration or the intravenous injection of 1.0 M NaHCO₃ solution. Oesophageal temperature was recorded and maintained at 37 ± 1°C (range) by heating lamps above and a thermostatically controlled heating blanket below the animal.

The ECG lead II was recorded and the signal used to drive a cardi tachometer. Systemic arterial pressure was recorded through a cannula inserted into the left femoral artery. All pressures were measured with strain gauge manometers (Hewlett Packard 4-442-001) attached to d.c. bridge amplifiers (Electromed). Blood flow in the left circumflex coronary artery was measured with either an electromagnetic flowmeter (Carolina Instruments Inc.) or an ultra-sound flowmeter (Transonic Inc.). The ECG, heart rate (HR), systemic arterial pressure and the mean and pulsatile coronary artery blood flow were recorded with a thermal array recorder (Gould TA4000).

¹ Author for correspondence at: H.M. Snow, Zeneca Limited, Research Department, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG.

Surgical procedures

The chest was opened in the 5th intercostal space, the left phrenic nerve divided and the pericardium opened to expose the left circumflex artery and vein. The circumflex coronary artery was damaged and stenosed as described by Folts *et al.* (1976). Briefly, about 2 cm of the left circumflex coronary artery was dissected free of surrounding tissue and the endothelium of a 5 mm length of artery damaged by crushing with a pair of Spencer Wells forceps. The damaged section of the artery was stenosed by a stiff plastic cuff which occluded the artery by about 60%. A flow probe was placed proximal to and a snare distal to the stenosis. Fine adjustment of the degree of stenosis was accomplished by passing a tapered nylon fishing line through the cuff. The degree of stenosis was adjusted until a 20 s occlusion with the distal snare did not produce a reactive hyperaemia, i.e. the distal vascular bed was fully dilated and therefore changes in blood flow (at constant heart rate and blood pressure) indicated changes in resistance at the stenosis.

In the above preparation the combination of stenosis and damaged endothelium induces platelet aggregation and the formation of a thrombus, increasing the resistance to blood flow. When blood flow approached zero, thrombi embolised either spontaneously or after gentle vibration of the plastic cuff; blood flow and thrombus formation then restarted and a pattern of cyclic flow reductions (CFRs) began, e.g. Figure 3 (Folts *et al.*, 1976; Folts, 1982).

Experimental protocol

In 10 dogs cumulative intravenous doses of the selective 5-HT₂ antagonist ICI 170809 (Blackburn *et al.*, 1990) in the range 0.1–100 µg kg⁻¹ were given at 10 min intervals. Heart rate, arterial blood pressure and coronary blood flow were recorded throughout and measurements made 10 min after each dose.

In 10 dogs the effects of infusions of adrenaline (range 0.2–20 µg kg⁻¹ min⁻¹, i.v.) on CFRs were obtained in the absence and presence of cumulative doses of ICI 170809 (range 1–1000 µg kg⁻¹, i.v.). In five of these dogs samples of arterial blood were taken 10 min after the start of adrenaline infusion for measurement of adrenaline concentration.

Adrenaline assay

The concentration of adrenaline in the plasma was assayed as described by Smith & Betteridge (1984) and Smith *et al.* (1985). Blood was taken into EDTA (1 mg ml⁻¹) and centrifuged immediately, the plasma was removed and stored at –70°C. Following thawing, centrifugation was performed to

Figure 1 The measured plasma concentration of adrenaline ($\pm$ s.e.mean) as a function of the rate of infusion of adrenaline.

remove fibrous protein and 1.5 ml of plasma added to an LP3 tube containing 10–20 mg of acid alumina; 10 µl BHBA (equivalent to 1000 pg) internal standard was then added, together with 1 M Tris buffer, pH 8.6. After shaking, mixing and washing of the alumina, 110 µl of eluent (acetic acid, EDTA, sodium disulphite solution) was added. After centrifugation 75 µl of alumina-free supernatant was assayed by high performance liquid chromatography (h.p.l.c.).

Figure 2 Records of mean and pulsatile blood flow in the stenosed left circumflex coronary artery showing the effects of adrenaline on the rate of thrombus formation as indicated by the rate of decline of coronary blood flow. Increasing the rate of adrenaline infusion to 0.2 (b) and 0.4 (c) µg kg⁻¹ min⁻¹, i.v. had no effects on blood pressure and heart rate; the rate of decline of blood flow was increased from 9.0 (a) to 11.4 (b) and 16.2 (c) ml min⁻².

Drugs

The following were used: adrenaline bitartrate (Sigma UK); ICI 170809 2-[3-(dimethylthylamino)ethyl]thio]-3-phenylquinoline (Zeneca Limited). All drugs were dissolved in sterile pyrogen-free 0.85% sodium chloride solution.

Results

About 1 h after the completion of surgical procedures, reproducible records of CFRs were usually obtained. In nine dogs, analysis of variance gave a within-dog standard error of the mean control estimate of the rate of decline of coronary blood flow of $\pm 6\%$ (Snedecor & Cochran, 1989).

When control CFR's were obtained in all 19 dogs the heart rate and mean systemic arterial blood pressure were respectively: 161 ± 20 beats min^{-1} (mean $\pm$ s.d.) and 120 ± 10 mmHg. The pH, PCO_2 and PO_2 of the arterial blood were respectively: 7.394 ± 0.05 , 37.4 ± 4.0 mmHg and 196 ± 26 mmHg (mean $\pm$ s.d.). The oesophageal temperature was $38.0 \pm 0.3^\circ\text{C}$.

The mean curve relating the infusion rate of adrenaline to the plasma concentration of adrenaline is shown in Figure 1. During the control period a measurable plasma concentra-

tion of adrenaline (0.2 nM) was found in only one dog. The two lowest rates of adrenaline infusion (0.2 and $0.4 \mu\text{g kg}^{-1} \text{min}^{-1}$) produced plasma concentrations of 2.16 ± 0.2 nM and 4.04 ± 0.61 nM (mean $\pm$ s.d.). At these levels, after an initial transient fall in blood pressure and increase in heart rate, no significant changes from control value were observed. At higher rates of infusion (mean plasma concentration above 7.64 ± 1.38 nM) arterial blood pressure increased with variable changes in heart rate.

The effects of intravenous infusions of adrenaline on blood flow in the stenosed circumflex coronary artery in one dog is shown in Figure 2. Ten minutes after the start of each infusion, the rate of decline of coronary blood flow (the index of the rate of thrombus formation) was increased from 9.0 ml min^{-2} to 11.4 ml min^{-2} and 16.2 ml min^{-2} . Similar results were obtained in 10 dogs; the control decline in blood flow was $8.7 \pm 1.4 \text{ ml min}^{-2}$ and was decreased to $12.0 \pm 1.5 \text{ ml min}^{-2}$ and $14.2 \pm 2.2 \text{ ml min}^{-2}$ respectively.

Records of coronary blood flow in one dog (Figure 3), in the absence of an infusion of adrenaline, show the effects of antagonism of the 5-HT_2 receptor by ICI 170809. The rate of thrombus formation was progressively reduced and at a dose of $10 \mu\text{g kg}^{-1}$, i.v., spontaneous embolization occurred and further thrombus formation ceased. Similar results were obtained in 10 dogs and the mean dose-response curve is shown in Figure 4. We normalized the response in each dog; complete prevention of thrombus formation was taken as 100% response. The mean ED_{50} for this initial effect of 5-HT_2 antagonism in the absence of adrenaline was $0.41 \mu\text{g kg}^{-1}$, i.v. (95% CI 0.2–0.8). The variation of the ED_{50} between individual dogs was large with an overall range from a minimum of 0.1 to $5.0 \mu\text{g kg}^{-1}$, i.v. The slope of the dose-response curve is unusually steep, from threshold to maximum effect takes place over a dose-range of less than one order of magnitude.

The interaction between 5-HT and adrenaline on thrombus formation was investigated by obtaining dose-response curves to adrenaline in the absence and in the presence of increasing doses of the 5-HT_2 antagonist, ICI 170809. In the presence of the 5-HT_2 receptor antagonist, increased amounts of adrenaline are required to restore equivalent rates of thrombus

Figure 3 Records of mean and pulsatile coronary blood flow in the stenosed left coronary artery showing the effects of antagonism, of the platelet 5-HT_2 receptor by ICI 170809. The doses of 2 (b) and $5 \mu\text{g kg}^{-1}$, i.v. (c) successively reduced the decline of blood flow from 28.0 (a) to 15.9 (b) and 3.2 (c) ml min^{-2} . After $10 \mu\text{g kg}^{-1}$, i.v. (c) spontaneous embolization occurred and thrombus formation was abolished.

Figure 4 Dose-response curve obtained in nine dogs relating the dose of ICI 170809 ($\mu\text{g kg}^{-1}$ i.v., mean $\pm$ s.e.mean) to the percentage change in the rate of decline of coronary blood flow (% change in slope). The curve was obtained by averaging the doses required to give the indicated responses, by interpolation on each individual dose-response curve.

Figure 5 Dose-response curves obtained in one dog, in the absence and presence of increasing doses of ICI 170809 (●, control; □ 10; ▲ 50; ○ 70; △ 200 and ■ 500 $\mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) relating the infusion rate of adrenaline ($\mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) to the rate of decline of coronary blood flow (ml min^{-2}) as an index of thrombus formation. Doses of 10 and 50 $\mu\text{g kg}^{-1}$, i.v., cause a rightward shift of the dose-response curve; 70–200 $\mu\text{g kg}^{-1}$, i.v., have no further detectable effect.

formation. However in this particular experiment, at doses of ICI 170809 of 50 $\mu\text{g kg}^{-1}$, i.v. and above no further effects on the adrenaline dose-response curve occurred. This inhibitory effect of ICI 170809 on the ability of adrenaline to increase the rate of thrombus formation in four dogs is shown in Figure 5. In each dog the dose-ratio (DR) of adrenaline measured at the same rate of thrombus formation, was calculated and plotted as $\log(\text{DR}-1)$ against the dose of ICI 170809. All the slopes are less than the theoretical slope of one for competitive inhibition, as might be expected for an interaction between adrenaline and 5-HT, acting as they do at different receptor sites. There is a tendency for the inhibitory effects of ICI 170809 to remain constant at doses above 50 $\mu\text{g kg}^{-1}$, i.v., at which dose the DR increase to adrenaline was 39 fold (12–126) (mean $\pm$ 95% CI).

In five dogs the effects of antagonism of the 5-HT₂ receptor on thrombus formation were obtained after the inhibition of the enzyme, cyclo-oxygenase, by aspirin (5 mg kg^{-1} , i.v.), thus removing the effects of arachidonic acid metabolites on platelet aggregation. Aspirin alone abolished thrombus formation which was then restarted by an infusion of adrenaline (4.0 $\mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.; plasma concentration 53.1 ± 13.9 nM, mean $\pm$ s.d.), an infusion rate which caused significant increases in arterial pressure. In these circumstances, ICI 170809 was still capable of preventing thrombus formation at doses of 50–100 $\mu\text{g kg}^{-1}$, i.v.

Discussion

This study shows that ICI 170809, a selective 5-HT₂ antagonist lacking effects at adrenoceptors (Blackburn *et al.*, 1988; 1990), inhibits both thrombus formation and also the potentiating effects of adrenaline, thereby implicating 5-HT in platelet aggregation.

The low plasma concentration of 5-HT coupled with low potency as a platelet aggregatory agent *in vitro* has raised doubts about the role of platelet-derived 5-HT in pathological states involving platelet aggregation (Robertson & Andrews, 1961; De Clerck & Herman, 1983; Van Neuton *et al.*, 1986).

However, 5-HT is concentrated in dense granules in the platelet and is released on activation, resulting in a relatively high concentration of 5-HT at the site of platelet aggrega-

tion. Ashton *et al.* (1986) measured an 18 fold increase in 5-HT concentration between damaged and normal sections of the left circumflex coronary artery of the dog. Also 5-HT markedly amplifies the effects of other agonists, such as ADP, adrenaline, noradrenaline, collagen and thromboxane A₂ (TXA₂), as demonstrated by De Clerck *et al.* (1984), using the 5-HT₂ antagonist, ketanserin. Furthermore there is some evidence that disease processes increase the sensitivity of tissues to 5-HT and that plasma 5-HT concentration is increased after myocardial infarction. Constituents of the atheromatous plaque, such as low density lipoprotein, have been reported to amplify 5-HT-induced platelet aggregation (Fetkovska *et al.*, 1989) and endothelial damage has been shown to enhance arterial contractile responses to 5-HT (Orlandi *et al.*, 1990).

In our experiments the steepness of the antagonist dose-response curve, in the absence of an adrenaline infusion (Figure 4) suggests that activation of only a small proportion of the available 5-HT₂ receptors is required to cause aggregation. These observations, demonstrating the importance of 5-HT in the formation of a platelet thrombus, are supported by clinical evidence that ketanserin may be effective for the prevention of intracoronary events in patients with ischaemic heart disease (Noble & Drake-Holland, 1990).

It has been demonstrated that adrenaline potentiates, and aspirin, thromboxane receptor antagonists, thromboxane synthase inhibitors and 5-HT₂ antagonists all inhibit thrombus formation in this dog model of coronary artery thrombosis (Sheng-Kun Yao *et al.*, 1990; McAuliffe *et al.*, 1992). In experiments reported here and elsewhere (Cox *et al.*, 1991a,b), it was shown that the effects of all of the above agents, whether present alone or in combination, can eventually be overcome by increasing the stimulus to thrombus formation in terms of adrenaline, degree of stenosis and endothelial damage.

Adrenaline is able to cause platelet aggregation directly and to potentiate the effects of other agonists (Grant & Scrutton, 1979; Peety & Scrutton, 1989). In the present experiments both mechanisms could have been responsible for the effects of adrenaline. The stepwise removal of the effects of 5-HT, caused by increasing doses of the 5-HT₂ antagonist, resulted in a rightward shift in the adrenaline dose-response curve (Figure 5), demonstrating the synergism between adrenaline and 5-HT on platelet aggregation and

Figure 6 The effect of ICI 170809 ($\mu\text{g kg}^{-1}$, i.v.), in four dogs, on adrenaline dose-response curves (see Figure 5); measured as the dose ratio (DR) of adrenaline required to give equal rates of decline of coronary blood flow (plotted as $\log \text{DR}-1$). In all four dogs the slope of the relationship is less than one. At doses above 50–100 $\mu\text{g kg}^{-1}$, i.v., ICI 170809 tends to become less effective in antagonizing the effects of adrenaline.

thrombus formation. However the maximum effects of 5-HT₂ antagonism were limited to about a 30 fold increase in the plasma concentration of adrenaline (Figure 6), such that doses of ICI 170809 above 100 µg kg⁻¹, i.v., had no further antagonistic effects on the ability of adrenaline to cause platelet aggregation. Assuming that there is an upper limit to the amount of 5-HT released by platelets on activation, it should be possible to antagonize completely the effects of 5-HT with the highest doses of ICI 170809 used in this study. Thus the inability to prevent platelet aggregation, in the presence of a high concentration of adrenaline, is likely to be the result of both the direct effect of adrenaline and synergism with other aggregants, e.g. TXA₂ and platelet activating factor (PAF). In a previous study (Torr *et al.*, 1990), another specific 5-HT₂ antagonist, ritanserin, appeared to antagonize completely the effects of infused adrenaline. A similar result (Belcher *et al.*, 1992) has been obtained for the 5-HT₂ receptor antagonist, MDL 11,939 (Dudley *et al.*, 1988). In these studies the maximum rate of infusion of adrenaline was only 0.4 µg kg⁻¹ min⁻¹, a rate of infusion much less than the maximum rate used in the present study.

The present approach has provided a more detailed and

quantitative examination of the interaction between 5-HT and adrenaline in coronary artery thrombus formation and shown that in an experimental situation plasma concentrations of adrenaline, well above the normal physiological range, are required to cause platelet aggregation *in vivo* after the removal of the effects of 5-HT.

Recently we have shown that the amount of adrenaline required to offset the maximum effects of aspirin, ICI 192605 a thromboxane A₂ antagonist and ICI 170809 are all similar (Snow *et al.*, 1992). Further the anti-aggregating effects of a 5-HT₂ antagonist and aspirin are additive, whereas those of a cyclo-oxygenase inhibitor (aspirin) and any other drug acting on the arachidonic acid/thromboxane pathway such as a TXA₂ receptor antagonist or a TXA₂ synthase inhibitor are obviously not. These results allow the speculation that ICI 170809 may be of clinical use in the treatment of coronary artery thrombosis, since Blackburn *et al.* (1990) have shown that ICI 170809 inhibits aggregation of human platelets *ex-vivo*, indicating that the effects of a specific 5-HT₂ antagonist in patients with a coronary thrombosis may be similar to those suggested for ketanserin (Noble & Drake-Holland, 1990).

References

- ASHTON, J.H., BENEDICT, C.R. & FITZGERALD, C. (1986). Serotonin as a mediator of cyclical flow variations in stenosed canine coronary arteries. *Circulation*, **73**, 572–578.
- ASHTON, J.H., GOLINO, P., MCNATT, J.M., BUJA, L.M. & WILLERSON, J.T. (1989). Serotonin S₂ and thromboxane A₂ prostaglandin H₂ receptor blockade provide protection against epinephrine induced cyclic flow variations in severely narrowed canine coronary arteries. *J. Am. Coll. Cardiol.*, **13**, 755–763.
- 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. *Cardiovascular Res.*, (in press).
- BLACKBURN, T.P., HAWORTH, S.J., JESSUP, C.L., MORTON, P.B. & WILLIAMS, C. (1990). ICI 170809, a selective 5-hydroxytryptamine antagonist, inhibits human platelet aggregation *in vivo* and *ex-vivo*. In *Cardiovascular Pharmacology of 5-hydroxytryptamine*. ed. Saxena, P.R., Wallis, D.I., Wouters, W. & Bevan, P. pp. 459–463. Kluwer: Academic Publishers.
- BLACKBURN, T.P., THORNBURGH, C.W., PEARCE, R.J. & COX, B. (1988). In-vitro pharmacology of ICI 170809 – a new 5-HT₂ antagonist. *FASEB*, **A1404**, 6441.
- COX, B., MCAULIFFE, S.J.G., NOBLE, M.I.M. & SNOW, H.M. (1991a). Inhibition of coronary artery thrombosis in the anaesthetized dog by antagonism of the 5-HT₂ receptors. *J. Physiol.*, **438**, 54P.
- COX, B., MCAULIFFE, S.J.G., MOORS, J.A., NOBLE, M.I.M. & SNOW, H.M. (1991b). The effects of adrenaline and serotonin on the rate of formation of platelet thrombi in a stenosed coronary artery of the anaesthetized dog. *J. Physiol.*, **438**, 169P.
- DE CLERCK, F. & HERMAN, G.G. (1983). 5-Hydroxytryptamine and platelet aggregation. *Fed. Proc.*, **42**, 228–232.
- DE CLERCK, F., XHONNEUX, B. & DE COURCELLES, D. (1988). Functional expression of the amplification reaction between serotonin and epinephrine on platelets. *J. Cardiovasc. Pharmacol.*, **11**, S1–5.
- DE CLERCK, F., XHONNEUX, B., LEYSEN, J.E. & JANNSEN, P.A.J. (1984). Evidence for functional 5-HT₂ receptor sites on human blood platelets. *Biochem. Pharmacol.*, **33**, 2808–2811.
- DUDLEY, M.W., WIECH, N.L., MILLERE, F.P., CARR, A.A., CHENG, H.C., ROEBEL, L.E., DOHERTY, N.S., YAMAMURA, H.I., URSILLO, R.C. & PALFREYMAN, M.G. (1988). Pharmacological effects of MDL 11,939: a selective, centrally acting antagonist of 5HT₂ receptors. *Drug Development Res.*, **13**, 29–43.
- EICHORN, E.J., GRAYBURN, P.A., WILLARD, J.E., ANDERSON, H.V., BEDOTTO, J.B., CARRY, M., KAHN, J.K. & WILLERSON, J.T. (1991). Spontaneous alterations in coronary blood flow velocity before and after coronary artery angioplasty in patients with severe angina. *J. Am. Coll. Cardiol.*, **17**, 43–52.
- FETKOVSKA, N., FETKOVSKA, V. & DZURIK, R. (1989). Low density lipoprotein amplifies the platelet response to serotonin in human plasma. *J. Hypertens.*, **7** (suppl. 6), S164–165.
- FOLTS, J.D. (1982). Experimental arterial platelet thrombosis, platelet inhibitors, and their possible clinical relevance. *Cardiovasc. Rev. Rep.*, **3**, 270–382.
- FOLTS, J.D., CROWELL, E.B. & ROWE, G.G. (1976). Platelet aggregation in partially obstructed vessels and its elimination with aspirin. *Circulation*, **54**, 365–370.
- GRANT, J.A. & SCRUTTON, M.C. (1979). Novel alpha₂-adrenoceptors primarily responsible for inducing human platelet aggregation. *Nature*, **277**, 659–661.
- HAEREM, J.W. (1972). Platelet aggregates in intramyocardial vessels of patients dying suddenly and unexpectedly of coronary artery disease. *Atherosclerosis*, **15**, 199–213.
- MCAULIFFE, S.J.G., SNOW, H.M., MOORS, J.A., WAYNE, M. & NOBLE, M.I.M. (1992). Inhibition of arachidonic acid metabolism by aspirin can promote thrombus formation in a stenosed coronary artery of the anaesthetized dog. *J. Physiol.*, **446**, 413P.
- NOBLE, M.I.M. & DRAKE-HOLLAND, A.J. (1990). The involvement of serotonin in the formation of thrombi at critical stenoses in humans. *Coronary Artery Dis.*, **1**, 675–679.
- ORLANDI, C., HUMPHREY, W.R., HOLLENBERG, N.K. & SCHAUB, R.G. (1990). In-vivo demonstration of enhanced platelet arterial constrictor response to serotonin following local endothelial loss. *Exp. Mol. Pathol.*, **52**, 192–201.
- PEETY, A.C. & SCRUTTON, M.C. (1989). Platelet aggregation in whole blood: is the response to adrenaline, 5-hydroxytryptamine and PAF a direct consequence of stimulation by these agonists? *Thrombosis Res.*, **54**, 151–159.
- ROBERTSON, J.I.S. & ANDREWS, T.M. (1961). Free serotonin in human plasma: quantitative and qualitative estimation. *Lancet*, **1**, 578–580.
- SHENG-KUN YAO, ROSOLOWY, M., ANDERSON, H.V., GOLINO, P., MCNATT, J.M., DE CLERCK, K., BUJA, L.M., WILLERSON, J.T. (1990). Combined thromboxane A₂ synthase inhibition and receptor blockade are effective in preventing spontaneous and epinephrine induced canine coronary cyclic flow variations. *J. Am. Coll. Cardiol.*, **16**, 705–713.
- SMITH, C.C.T. & BETTERIDGE, D.J. (1984). Improved liquid chromatographic determination of catecholamines in platelets. *Clin. Chem.*, **30**, 1432–1433.
- SMITH, C.C.T., CURTIS, L.D., DELAMONTE, A.P., PRITCHARD, B.N.C. & BETTERIDGE, D.J. (1985). The distribution of catecholamines between platelets and plasma in normal human subjects. *Clin. Sci.*, **69**, 1–6.
- SNEDECOR, G.W. & COCHRAN, W.G. (1989). Statistical Methods. 8th edition. Iowa State University Press, Ames, Iowa.
- SNOW, H.M., MCAULIFFE, S.J.G., JESSUP, R., WAYNE, M. & NOBLE, M.I.M. (1992). Prevention of coronary thrombosis in the dog by inhibitors of platelet aggregation. *Br. J. Pharmacol. Proc. Suppl.* (in press).

TORR, S., NOBLE, M.I.M. & FOLTS, J.D. (1990). Inhibition of acute platelet thrombosis formation in the stenosed canine coronary arteries by the specific serotonin 5-HT₂ receptor antagonist ritanserin. *Cardiovasc. Res.*, **24**, 465–470.

VAN NEUTON, J.M., XHONNEUX, R. & JANSSENS, W.J. (1986). Interaction between S₂-serotonergic and alpha₁-adrenergic receptors and the control of blood pressure. In *Mechanisms of Vasodilatation IV*. Rochester, Minn.

(Received December 8, 1992

Revised January 26, 1993

Accepted January 29, 1993)