The effects of dopexamine, a new dopamine analogue, on platelet function in stress

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1 Dopexamine is a novel analogue of dopamine which is free of α -adrenoceptor activity and is of therapeutic value in chronic heart failure. The effects of dopexamine on the *in vitro* function of platelets from 10 healthy subjects at rest, after exercise and after *in vitro* addition of adrenaline and noradrenaline were investigated.

2 Dopexamine in a wide range of concentrations (10^{-9} M– 10^{-3} M) did not appear to function as an agonist on platelets either in whole blood or in PRP preparations.

3 Dopexamine caused a dose-dependent inhibition of agonist-induced platelet aggregation in both whole blood and PRP. The inhibitory effect of dopexamine was significantly greater in PRP than in whole blood, and significantly greater to adrenaline than to collagen or ADP as agonists in whole blood.

4 After exercise or after *in vitro* addition of adrenaline and noradrenaline at concentrations commonly seen in myocardial infarction, dopexamine produced similar levels of inhibition seen with platelets from resting subjects.

5 Dopexamine did not affect plasma catecholamine levels but caused an increase in intraplatelet noradrenaline levels.

6 This study suggests that dopexamine is unlikely adversely to affect the hyperaggregable state found in patients with cardiogenic shock after myocardial infarction.

Keywords dopexamine platelets stress dopamine

Introduction

Inotropes and vasodilators are used to treat heart failure resistant to conventional diuretic therapy (Goldberg, 1974; Miller *et al.*, 1981). The catecholamine dopamine may be of value in the management of chronic heart failure (Loeb *et al.*, 1977), but its α -adrenoceptor agonist activity has limited its use (Brown *et al.*, 1985a). Dopexamine is a new dopamine analogue which is as potent as dopamine in increasing renal blood flow but with different systemic effects (Brown *et al.*, 1985b). It acts as an agonist at peripheral vascular dopamine (DA_1)-receptors and has mild prejunctional DA_2 -receptor ac-

tivity. It is a potent β_2 -adrenoceptor agonist but has no α -adrenoceptor activity. The compound also inhibits neuronal re-uptake of catecholamines (Svenson *et al.*, 1988), so it may affect agonist receptor interactions in this fashion (Jaski *et al.*, 1986). Furthermore it has no appreciable β_1 -receptor activity (Brown *et al.*, 1985a; Heitz *et al.*, 1983) and evidence suggests it has potent inotropic properties probably via myocardial β_2 -adrenoceptors (Miller *et al.*, 1981).

The acute (Dawson *et al.*, 1985; Svensson *et al.*, 1986) and long-term (Murphy & Hampton,

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1988) effects of dopexamine in chronic heart failure have been described: it produces a fall in systemic vascular resistance and increases cardiac output. No alteration in the mean arterial pressure and no significant changes in myocardial oxygen consumption or metabolic function have been observed. Dopexamine appears to be unable to maintain these haemodynamic improvements in severe chronic heart failure after 18 h of infusion (Murphy & Hampton, 1988); however its efficacy is reported to be well-maintained in mild heart failure following acute myocardial infarction (Svenson *et al.*, 1988). These observations suggest that dopexamine may be of value in cardiogenic shock.

Cardiogenic shock is the major cause of death in hospital after myocardial infarction. It has been demonstrated that the fate of the coronary artery thrombus has a major effect on cardiac function, with spontaneous or pharmacologically-induced recanalisation producing improvements in left ventricular ejection fraction (Ong *et al.*, 1983; The ISAM Study Group, 1986) and persistent occlusion being found in patients in cardiogenic shock (De Wood *et al.*, 1980). Previous work from this unit has shown that patients with cardiogenic shock or pulmonary oedema after myocardial infarction, exhibit marked platelet activation related to the increase in the levels of adrenaline and glucose (Oswald *et al.*, 1988). This may be a similar mechanism to the platelet hyperaggregability and activation which has been demonstrated after physical exercise (Hendra *et al.*, 1988). It would thus appear that a vicious cycle of elevated levels of stress hormones and increased thrombogenesis, with concurrent failure of spontaneous thrombolysis, may occur after myocardial infarction, resulting in deteriorating cardiac function. If dopexamine were to stimulate platelet activation, it might produce further adverse effects in patients with cardiogenic shock.

The platelet has properties similar to the adrenergic neurone in terms of uptake and storage of catecholamines (Abrams & Solomon, 1969; Pletscher & Laubscher, 1980) and stress-induced platelet hyperaggregability has been ascribed, at least in part, to intraplatelet catecholamines (Wilson *et al.*, 1987). It is possible that dopexamine may affect platelet aggregability by inhibiting platelet storage of catecholamines; alternatively, if circulating levels of catecholamines were increased because of diminished neuronal uptake, platelet activation could result. A final pathway whereby dopexamine might affect platelet aggregation is through a possible inhibiting effect via its β_2 -adrenoceptor agonist activity (Winther, 1987).

The present study was undertaken to assess the *in vitro* effects of dopexamine on *in vitro* platelet function at rest, and after exercise, using a bicycle ergometer to raise catecholamines to the levels found in stress. The effects of dopexamine were assessed using three measures of *in vitro* platelet function:

1. Agonist-induced aggregation in platelet-rich-plasma (PRP) using a standard light-scattering technique (Born, 1962);
2. Spontaneous aggregation in agitated whole blood (Hendra *et al.*, 1988); and
3. Agonist-induced aggregation in whole blood (Lumley & Humphrey, 1981).

Spontaneous platelet aggregation was not studied in PRP because it occurs infrequently in normal subjects (Kardinal *et al.*, 1975) and is demonstrable for only a short time after preparation of PRP (Wu & Hoak, 1976). The study was designed to test the following hypotheses:

1. Since dopexamine has less α_2 -adrenoceptor activity than dopamine, it is unlikely to stimulate platelet activation.
2. Dopexamine inhibits platelet catecholamine uptake.

Methods

Ten healthy subjects aged 22–48 (median 31) years, and without clinical evidence of cardiovascular disease, were studied. None of the subjects had taken any drugs known to affect platelet function for the previous 14 days. All were studied in the morning in the fasting state, and without smoking on the day of the study.

Study 1

Venous blood samples were taken without stasis, using a 19 gauge needle, from the antecubital vein. Plasma and platelet adrenaline and noradrenaline levels were measured and platelet studies were carried out (*vide infra*) on blood samples incubated for 30 min with saline (controls) or dopexamine at various concentrations.

Study 2

Each subject exercised on a Tunturi bicycle ergometer (Tunturi, Finland) for 24–28 min to achieve an exercise end-point of exhaustion, visible beads of perspiration and a pulse rate of 160 beats min^{-1} (Hendra *et al.*, 1988). Immediately following exercise, blood samples were taken and platelet studies performed (*vide infra*) after incubation with saline or dopexamine.

Study 3

Resting blood samples were taken from the same subjects and incubated for 30 min with saline (controls) or adrenaline 2.0×10^{-9} M and noradrenaline 10^{-8} M to simulate the levels of catecholamines found in patients after myocardial infarction (Oswald *et al.*, 1988). To these samples dopexamine was added as before and platelet studies were again carried out. These studies were completed in only eight of the ten subjects.

Platelet studies

(a) *Platelet aggregation in platelet rich plasma (PRP)* Platelet aggregation in PRP was studied using the method of Born (1962). Venous blood was anticoagulated with 3.8% trisodium citrate in a ratio of 9:1 v/v. All platelet counts were corrected to $300 \times 10^9 \text{ l}^{-1} \pm 15 \times 10^9 \text{ l}^{-1}$ using autologous platelet poor plasma (PPP). Platelet aggregation was induced, at $37^\circ \text{C} \pm 0.2^\circ \text{C}$, by addition of each of four concentrations of ADP (10^{-6} M, 3×10^{-6} M, 10^{-5} M and 10^{-4} M), adrenaline (10^{-6} M, 3×10^{-6} M, 10^{-5} M and 10^{-4} M) (both from Sigma Chemicals Co, Poole, Dorset, UK) and collagen ($0.1 \mu\text{g ml}^{-1}$, $0.3 \mu\text{g ml}^{-1}$, $1 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$) (Hormon Chemi, Munich, FRG) in cuvettes with a stirrer (Sartec Ltd, Sevenoaks, Kent, UK). The aggregation response to each agonist concentration was measured as the change in light transmission 5 min after addition of the agonist. In addition the effect of dopexamine alone as a putative platelet agonist was assessed on the control blood samples, in concentrations ranging from 10^{-9} M to 10^{-3} M. In four subjects, no secondary aggregation occurred in response to adrenaline in any concentration.

(b) *Platelet aggregation in whole blood* This was assessed using the Ultraflo platelet counting technique (Lumley & Humphrey, 1981) and the same four concentrations of each agonist. Aliquots of the citrated blood with added saline (controls) or dopexamine were shaken under 5% CO_2 in air in a waterbath, controlled thermostatically at $37^\circ \text{C} \pm 0.2^\circ \text{C}$, for 30 min during which time the platelet count in each aliquot stabilised. Platelet aggregation responses were determined by measuring the percentage fall in platelet count in each aliquot after addition of the agonist. The fall in platelet count was recorded at 3 min for ADP and 5 min for adrenaline and collagen, these times having been previously determined to produce the most reproducible aggregation response times (Hendra *et al.*,

1988). Two subjects showed no aggregation in response to adrenaline in any concentration.

(c) *Spontaneous platelet aggregation in whole blood* This was assessed in the same way as for whole blood aggregation, with addition of saline or of dopexamine, except that no agonists were added, and platelet counts were determined at times 0, 10, 20, 30 and 60 min during agitation at 37°C (Hendra *et al.*, 1988). These studies were performed on samples from five to eight subjects with each concentration of dopexamine and for each experimental condition. Because all whole blood and PRP studies after exercise and after addition of adrenaline and noradrenaline had to be completed in a limited time period, only two concentrations of dopexamine were employed for these studies.

Measurement of plasma and platelet catecholamine levels

Plasma and platelet catecholamines were measured in venous blood samples collected into EDTA. After addition of either saline or dopexamine blood samples were incubated for 30 min before centrifugation at 200 g for 10 min at room temperature. Platelets were harvested from the PRP so obtained by centrifugation at 2000 g for 20 min at 4°C . The supernatant PPP was removed and stored on ice and the platelet pellets washed twice with a buffered Tyrode solution. Platelet pellets were then stored at -80°C . Blood from which the PRP was obtained was recentrifuged at 2000 g for 15 min to obtain more PPP. The two samples of PPP were pooled and stored in aliquots at -80°C . At the time of analysis platelet pellets were resuspended in 1 ml of 0.1 M perchloric acid and homogenised in a ground-glass homogeniser together with 50 μl of 3,4 dihydroxybenzylamine internal standard. The homogenates were then centrifuged at 12,000 g for 5 min. The perchloric acid supernatants were separated from the pellets and the supernatant and thawed plasma samples analysed for catecholamines by high performance liquid chromatography with electrochemical detection (Smith *et al.*, 1985). The platelet pellets were assayed for protein (Lowry *et al.*, 1951).

The protocol for this study was approved by the Islington District Ethics Committee.

Statistical analyses of results

Percent spontaneous aggregation or percent inhibition of platelet aggregation was calculated. Saline control values were compared with the

corresponding values obtained following incubation with dopexamine for each experimental condition. For agonist-induced platelet aggregation in PRP and whole blood, EC_{50} s were calculated graphically for each concentration of dopexamine and the reciprocals of the values so obtained were used to calculate percentage sensitivity by comparison with the reciprocal of the EC_{50} of the saline control, which was taken to correspond to 100% sensitivity. The IC_{50} was calculated as the concentration of dopexamine producing a twofold increase in agonist EC_{50} by plotting the reciprocal of EC_{50} against dopexamine concentration. Paired Student's *t*-tests were used for parametric data and Wilcoxon's paired tests for non-parametric data. In some instances where different numbers of subjects were studied in different conditions, or where certain agonists failed to induce aggregation, these comparisons have been made only for those subjects in whom complete data are available. Results are expressed as mean (s.d.) for normally distributed data and as median (range) for skewed data. The numbers presented are either the number of subjects or the number of observations, combining different experimental conditions or time points as indicated in the text and tables.

Results

Dopexamine as an agonist and antagonist of platelet aggregation

Initial experiments were conducted to establish whether dopexamine functions as a platelet agonist or as an inhibitor of platelet aggregation induced by the three agonists. The concentrations of dopexamine employed ranged from 10^{-9} M to 10^{-3} M. No agonist effect was observed at any concentration in five subjects in either PRP or whole blood using four to seven concentrations in each assay system. Dopexamine at a concentration of 10^{-3} M completely blocked agonist-induced platelet aggregation in both PRP and whole blood. At a concentration near to or within the therapeutic range (10^{-9} M to 10^{-6} M), dopexamine had little or no inhibitory effects on agonist-induced platelet aggregation. The IC_{50} for dopexamine in studies of aggregation using PRP and whole blood techniques was assessed by performing full aggregation studies using three to six concentrations of dopexamine in three to six subjects with each agonist. However, adrenaline induced no aggregation in either whole blood or PRP in two of these subjects whether or not dopexamine was

Table 1 Calculation of IC_{50} for dopexamine in resting subjects

ADP	PRP		Collagen	Whole blood		Collagen
	Adrenaline			Adrenaline		
0.6×10^{-5}	4.0×10^{-6}		2.2×10^{-5}	2.8×10^{-5}		3.2×10^{-5}
$(1.6 \times 10^{-6}$	$(6.0 \times 10^{-8}$		$(1.0 - 3.0 \times 10^{-5})$; (6)	$(2.2 \times 10^{-6}$		$(1.5 - 9.0 \times 10^{-5})$; (5)
$- 1.4 \times 10^{-5})$; (5)	$- 1.4 \times 10^{-5})$; (3)		$- 1.2 \times 10^{-4})$; (5)	$- 3.0 \times 10^{-5})$; (3)		

Values are given as median, (range); (number), molar concentrations of dopexamine causing 50% maximum inhibition of agonist-induced platelet aggregation.

added to the samples. The median IC_{50} s in these subjects are presented in Table 1.

Spontaneous platelet aggregation

Figure 1 and Table 2 illustrate the effect of dopexamine on spontaneous platelet aggregation. Compared with spontaneous aggregation in saline, dopexamine in molar concentrations of

$10^{-6}M$, $3.0 \times 10^{-5}M$ and $3.0 \times 10^{-4}M$ caused dose-dependent reductions in aggregation. The median percentage reductions in aggregation at all time points are shown in Table 2 for each concentration of dopexamine, while Figure 1 illustrates the effect of dopexamine on the percent platelet aggregation under different experimental conditions. The proportional effect of dopexamine in reducing platelet aggregation

Figure 1 The effect of dopexamine on the percentage spontaneous platelet aggregation under different experimental conditions. (a) Resting subjects, (b) after exercise and (c) after *in vitro* addition of adrenaline and noradrenaline. (○) Saline control, (●) dopexamine $10^{-6}M$, (□) dopexamine $3 \times 10^{-5}M$, (■) dopexamine $3 \times 10^{-4}M$. The significance of the differences is indicated thus: * $P < 0.05$, ** $P < 0.01$, compared with saline controls.

Table 2 Percentage reduction in spontaneous platelet aggregation by dopexamine at different concentrations

Concentrations of dopexamine (M)	At rest	After exercise	After <i>in vitro</i> addition of adrenaline and noradrenaline
10^{-6}	12.3(−73.8 to 75);(20)	—	—
3×10^{-5}	13.8(−150 to 73.8);(28)	10.8(−68.2 to 40.2);(24)	24.0(−37.8 to 53.1);(32)
3×10^{-4}	54.6(0 to 100);(24) ^{ab}	31.5(−5.0 to 86.4);(24) ^b	49.8(−37.8 to 75.0);(28) ^b

Values are given as median, (range); (number of observations). The percentage reduction is shown for each concentration of dopexamine for all four time points combined. The studies were performed in five to eight subjects for each concentration of dopexamine and for each experimental condition.

^a $P < 0.001$ compared with dopexamine $10^{-6}M$

^b $P < 0.001$ compared with dopexamine $3 \times 10^{-5}M$

was not significantly different after exercise or after *in vitro* addition of adrenaline and noradrenaline compared with the inhibition in blood from resting subjects.

Whole blood and PRP platelet studies

As in our previous studies (Hendra *et al.*, 1988) exercise produced significant reductions in EC_{50} to adrenaline and collagen in whole blood. Pre-incubation with noradrenaline and adrenaline caused significant reduction in EC_{50} to ADP both in whole blood and PRP. Table 3 shows the effect of dopexamine on agonist-induced platelet aggregation in whole blood and PRP using between two and six concentrations of dopexamine in each subject. Dopexamine produced similar percentage inhibition of agonist-induced platelet aggregation under all experimental conditions in both whole blood and PRP and so the mean sensitivity to agonists at each dopexamine concentration is shown for all conditions combined. At concentrations of $3 \times 10^{-5}M$ and $3 \times 10^{-4}M$, dopexamine produced greater inhibition of aggregation in PRP than in whole blood for all three agonists ($P < 0.001$ – < 0.01 for the six comparisons). In PRP the degree of inhibition was, with one exception, similar for each agonist at each concentration of dopexamine, but in whole blood, dopexamine at concentrations of $3 \times 10^{-5}M$ and $3 \times 10^{-4}M$ produced greater inhibition to aggregation induced by adrenaline than to that induced by other agonists ($P < 0.002$ – < 0.05 for the four comparisons).

Platelet catecholamine levels

The effect of dopexamine on intraplatelet catecholamine levels is shown in Table 4. Dopexamine did not affect plasma catecholamine levels. However, comparison with results obtained for saline incubations, showed that it caused an increase in intraplatelet noradrenaline but not in adrenaline levels.

Discussion

Dopexamine over a wide range of concentrations ($10^{-9}M$ to $10^{-3}M$), including concentrations near to or within the therapeutic range, had no agonist effect on platelet aggregation in whole blood or PRP. However at the higher concentrations employed, dopexamine inhibited both spontaneous and agonist-induced aggregation. This effect is probably multifactorial but can be explained, at least partly, as follows. Firstly, dopexamine has less α_2 -

adrenoceptor agonist activity than dopamine (Brown *et al.*, 1985a) and therefore is less likely to stimulate platelet activation through decreases in intraplatelet cAMP levels (Bick, 1985; Fain & Garcia-Sainz, 1980). Secondly, dopexamine has marked DA_1 -receptor agonist activity. Stimulation of DA_1 -receptors activates adenylate cyclase (Shultz *et al.*, 1987) with consequent elevation of the levels of cAMP. Thus the net effect of the compound is likely to be an increase in the levels of cAMP resulting in inhibition of platelet aggregation by causing binding of intraplatelet ionised calcium (Bick, 1985). This action of dopexamine would be opposed by stimulation of DA_2 -receptors but since dopexamine has only mild DA_2 activity, this would be expected to be overcome by the much more marked DA_1 -receptor agonist activity of dopexamine (Brown *et al.*, 1985a). An alternative explanation for the inhibitory effects of dopexamine on platelet aggregation is via its β_2 -adrenoceptor agonist activity, the effect of which would be to elevate intraplatelet cAMP levels and thereby inhibit platelet aggregation (Winther, 1987). Differentiating these effects might be possible by the use of specific antagonists.

It is possible that dopexamine may have an additional action as an α_2 -adrenoceptor antagonist, which could be responsible for the greater inhibition observed to aggregation employing adrenaline as an agonist (see Table 3) (Grant & Scrutton, 1980). However, we observed no difference in percentage inhibition of aggregation by dopexamine after preincubation of platelets with catecholamines. These observations suggest that dopexamine would be likely to have similar effects on platelets in patients after myocardial infarction as in resting subjects.

Dopexamine caused an increase in intraplatelet noradrenaline levels. These results do not support the hypothesis that dopexamine inhibits platelet uptake and storage of catecholamines as it does in sympathetic neurons (Abrams & Solomon, 1969; Pletscher & Laubscher, 1980). This may mean that in this regard platelets behave differently from sympathetic neurons. It is possible that dopexamine acts by inhibiting catecholamine release by stimulation of DA_2 -receptors on platelets. Stimulation of this type of prejunctional receptor inhibits catecholamine release from sympathetic nerve endings (Brown *et al.*, 1985a). The lack of effect of dopexamine on plasma catecholamine levels excludes the possibility of cross-reaction of dopexamine in the h.p.l.c. system.

Thus in conclusion we have demonstrated that dopexamine had no agonist effects on platelets

Table 3 Effect of dopexamine in different concentrations on agonist-induced platelet aggregation in platelet-rich-plasma and whole blood

Agonist	Saline control	Dopexamine concentration							
		$10^{-8} M$	$10^{-7} M$	$10^{-6} M$	$10^{-5} M$	$3 \times 10^{-5} M$	$10^{-4} M$	$3 \times 10^{-4} M$	$10^{-3} M$
ADP Collagen Adrenaline	100%	66(16);(6)	91;(2)	58(27);(10)	51;(2)	27(20);(24)	7(10);(7)	1(2);(18)	0(0);(2)
	100%	48(22);(6)	81(54);(3)	71(17);(11)	58(28);(4)	32(18);(22)	10(21);(8)	0(0);(17)	0(0);(2)
	100%	60;(2)	47;(1)	63(48);(6)	14;(2)	9(12);(11) ^a	0(1);(4)	0(0);(8)	0;(1)
PRP									
ADP Collagen Adrenaline	100%	96;(1)	86(20);(3)	75(16);(11)	61(20);(5)	77(24);(24) ^b	32(28);(7)	46(21);(19) ^b	—
	100%	96;(1)	135;(1)	64(27);(9)	65;(2)	67(23);(23) ^b	29(15);(6)	36(25);(18) ^b	—
	100%	—	65;(1)	76(28);(7)	70(17);(4)	46(25);(16) ^{abc}	31(20);(7)	12(15);(14) ^{def}	0;(2)
Whole blood									

Values are expressed as mean (s.d.); (number of observations) and are given as percentage aggregation sensitivity, calculated as the reciprocal of the value for EC₅₀ at each concentration of dopexamine, and employing the saline control EC₅₀ for each subject and agonist as 100%. Between eight and ten subjects were studied at rest, after exercise, and with addition of adrenaline and noradrenaline, with EC₅₀s calculated for between two and seven concentrations of dopexamine for each sample. Four subjects showed no aggregation to adrenaline in PRP and two in whole blood.

^a $P < 0.05$ compared with sensitivity with collagen as agonist

^b $P < 0.001$ compared with sensitivity in PRP

^c $P < 0.01$ compared with sensitivity with ADP as agonist

^d $P < 0.01$ compared with sensitivity in PRP

^e $P < 0.01$ compared with sensitivity with collagen as agonist

^f $P < 0.002$ compared with sensitivity with ADP as agonist

Table 4 Effect of dopexamine on the levels of platelet and plasma adrenaline and noradrenaline

Saline/dopexamine	Plasma		Platelets	
	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline
Saline	0.19(0.16);(4)	2.37(0.26);(4)	0.08(0.05);(5)	1.92(0.80);(5)
3×10^{-4} M dopexamine	0.40(0.46);(5)	2.78(0.54);(5)	0.13(0.13);(5)	3.46(1.59);(5)*

Values are given as mean (s.d.); (number) and are expressed as pmol ml⁻¹ plasma and as pmol mg⁻¹ platelet protein.

* $P < 0.05$ compared with saline controls

from normal subjects and that at higher concentrations than achieved *in vivo* it caused a dose-dependent inhibition of agonist-induced platelet aggregation which was unaffected by an increase in plasma catecholamine levels. The efficacy of dopexamine in improving haemodynamics in chronic heart failure and in moderate heart failure after myocardial infarction has been demonstrated (Dawson *et al.*, 1985; Murphy &

Hampton, 1988; Svenson *et al.*, 1988; Svensson *et al.*, 1986). This study suggests that dopexamine is unlikely adversely to affect the hyperaggregable state found in patients with cardiogenic shock after myocardial infarction.

We thank Caroline Jackson for computing expertise and Sharrone McCullough for secretarial work. During the period of this study, Dr Wagaine-Twabwe was supported by grants from Fisons (UK) plc.

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(Received 3 July 1989,
accepted 19 February 1990)