Effects of meclofenamate and captopril on renal and other regional vascular beds after mild haemorrhage in conscious rabbits

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Summary

1. The role of prostaglandins and angiotensin II in blood flow regulation was studied in conscious rabbits subjected to mild haemorrhage.

2. Haemorrhage caused a 13% fall in arterial pressure and a 21% fall in cardiac output, responses which were unchanged by sodium meclofenamate, an inhibitor of prostaglandin synthesis, or captopril, an inhibitor of the angiotensin converting enzyme.

3. Haemorrhage doubled plasma adrenaline and noradrenaline levels. Plasma renin activity trebled after haemorrhage and was further elevated by captopril.

4. Renal blood flow was maintained after haemorrhage alone. Meclofenamate given immediately after haemorrhage caused a 31% fall in renal blood flow. Captopril given immediately after haemorrhage caused renal vasodilation, but when given after meclofenamate augmented renal vasoconstriction.

5. Splenic vasoconstriction was seen after haemorrhage and meclofenamate, and subsequently was augmented by captopril.

6. Results suggest that prostaglandins variably modulate regional blood flow in conscious rabbits subjected to mild haemorrhage. Enhanced sympatho-adrenal activity increases renovascular and splenic dependence on vasodilator prostaglandins, but not that of coronary, cerebral, hepatic or adrenal circulations. Renal and splenic vasoconstriction seen with meclofenamate are not due to circulating angiotensin II.

Key words: angiotensin, captopril, haemorrhage, kidney, meclofenamate, prostaglandins, spleen.

Introduction

In the normal conscious rabbit sodium meclofenamate and indomethacin, two inhibitors of prostaglandin cyclo-oxygenase, reduce renal blood flow and increase renal vascular resistance [1-3]. It has been suggested that this renal vasoconstriction is due to the unopposed action of neural or circulating vasoconstrictors, particularly where the activity of these factors is enhanced [4]. Thus indomethacin is ineffective in intact conscious dogs, but causes renal vasoconstriction after haemorrhage [5]. Similarly when dogs are anaesthetized the fall in renal blood flow after haemorrhage is greater after indomethacin [6]. Romero & Strong found that the fall in renal blood flow after chronic renal artery constriction was exaggerated by indomethacin, sometimes leading to oliguric renal failure [7]. Pugsley et al. reported an exaggerated rise in blood pressure in two-kidney, one-clip Goldblatt hypertensive rats treated chronically with indomethacin [8].

The mechanism of the enhanced renal vasoconstriction after inhibition of prostaglandin synthesis in the presence of haemorrhage remains unclear. Moreover the relative importance of prostaglandins in maintaining flow to organs other than the kidneys is uncertain. The object of

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these experiments was therefore further to evaluate the role of endogenous prostaglandins and of angiotensin II on organ blood flow in conscious rabbits subjected to mild haemorrhage.

Methods

Twenty-one male lop-eared rabbits weighing from 3.1 to 4.3 kg were used. Details of surgery, and of measurement of cardiac output and organ blood flow in the conscious rabbit, have been given before [2]. Briefly, animals were prepared under anaesthesia for cardiac output and organ flow measurements the day before an experiment by cannulation of the left ventricle and implantation of an aortic thermistor. On the day of the experiment the right ear artery was cannulated under local anaesthesia for blood flow measurements.

Measurements of cardiac output distribution were made by left ventricular infusion of 150 000 radioactively labelled microspheres (mean diameter 16.1 ± sd 1.1 μm). Total cardiac output was estimated from the mean of four or five thermodilution curves obtained by injections (0.7 ml) of sodium chloride solution (150 mmol/l: saline) into the left ventricle just before microsphere infusions. Spheres labelled with 52cobalt, 46scandium and 113tin were used for the three consecutive measurements.

Fifteen minutes after ear artery cannulation, when the rabbit sat quietly in the restraining box, the first set of thermodilution cardiac output measurements were made. Microspheres labelled with the first radioisotope were then infused into the left ventricle. These manoeuvres measured the pretreatment state. Three 1.0 ml blood samples were then withdrawn from the arterial cannula, the first and second were taken in iced tubes containing anticoagulant and preservative, for the estimation of plasma renin activity by radioimmunoassay and of plasma adrenaline and noradrenaline concentrations by radioenzymatic assay by the method of Peuler & Johnson [9] with the addition of external standards. The third blood sample was for blood gas analysis. The ear artery cannula was then connected to a syringe withdrawal pump (Sage Instruments, model 352) and by this route the animals were bled at a controlled rate of 1 ml/min to a total of 10 ml/kg body weight. This represented a body loss of approximately 15% of blood volume over 45–60 min. Blood pressure and pulse were measured at intervals during the haemorrhage. After removal of the calculated volume, blood samples for plasma renin activity and catecholamine estimations were repeated as above and blood pressure monitoring was continued.

The rabbits were then divided into three groups of seven in random order. In group 1, which acted as control, 5 min elapsed before bicarbonate buffer (1 ml/kg) was infused through a scalp vein needle in a marginal ear vein. The 5 min infusion was followed by a 10 min interval, after which a second set of thermodilution curves was recorded and microspheres labelled with the second isotope were infused into the left ventricle. This pattern of a 5 min interval, buffer infusion and a further 10 min interval was repeated before the final measurements with thermodilution curves and microspheres.

In group 2, haemorrhage and blood sampling were immediately followed by intravenous infusion of sodium meclofenamate (6 mg/kg). After 15 min, thermodilution measurements and a microsphere infusion were repeated. Ten minutes later captopril (1 mg/kg) was given intravenously over 5 min, and after a further 5 min the final set of haemodynamic observations was made by thermodilution and microspheres.

Rabbits in group 3 received the captopril infusion (1 mg/kg) 10 min after haemorrhage and 5 min later haemodynamic measurements were performed. Meclofenamate (6 mg/kg) was then given intravenously and the effects were measured after 15 min.

Thus in group 1, the effects of haemorrhage alone were measured whereas in groups 2 and 3 the additional effects of either meclofenamate or captopril alone and then in combination were recorded.

A dose of 6 mg of meclofenamate/kg was chosen as this dose has previously been shown by us to cause submaximal renal vasoconstriction in conscious rabbits [2] and has been reported to cause over 90% inhibition of rabbit renal medullary prostaglandin synthesis for over an hour after intravenous infusion [10].

At post mortem the position of cannula in the left ventricle was checked and organs were dissected, weighed and prepared for radioactivity counting. Organs studied were kidneys, heart, brain, spleen and adrenal glands.

Sodium meclofenamate was dissolved in a sodium bicarbonate buffer, pH 8.5, and captopril was in saline. Both solutions were prepared immediately before infusion. In each case the millilitre volume per dose was equal to the kilogram weight of the rabbit. The dose of captopril used in these experiments has previously been shown to inhibit the pressor response to a 300 μg/kg dose of angiotensin I by 97% and 89% at 5 and 25 min after ad-
ministration of the converting enzyme inhibitor [2].

Changes within groups were analysed by paired Student's t-test and those between groups by the one-way analysis of variance or non-paired t-test. A P value of <0.05 (two-tailed) was considered significant. Values in the text are means ± SD and in the Tables and Figures are means ± SEM.

Results

Haemodynamic experiments

Animals did not appear disturbed by microsphere infusions. Arterial blood pH was 7.45 ± 0.01 before and 7.43 ± 0.01 after haemorrhage. Mean blood loss volume was similar in each group (10.1, 10.0 and 10.3 ml/kg in groups 1, 2 and 3 respectively).

There were no significant differences between the three groups in pretreatment mean values of arterial pressure, cardiac output, organ blood flows, plasma renin activity or plasma catecholamines.

Effects on plasma pressor hormones

Plasma renin activities tripled with haemorrhage (Table 1). The subsequent change in plasma renin activity followed one of two distinct patterns, dependent on the nature of the first drug treatment. The plasma renin activity of rabbits in groups 1 and 2 fell after administration of buffer or sodium meclofenamate respectively. In contrast animals receiving captopril (group 3) demonstrated a further steep rise in plasma renin activity.

Plasma adrenaline and noradrenaline levels increased two- to three-fold immediately after haemorrhage in each group (Table 1).

Effects on systemic haemodynamics

Mean blood pressure did not fall below 55 mmHg at any time in any rabbit. In group 1 haemorrhage followed by first and second doses of buffer resulted in 13% and 9% reductions in mean arterial pressure respectively (Table 2). Pulse rate per minute increased from a mean of 244 to 272 (P < 0.05) after the first and to 291 (P < 0.01) after the second buffer infusion. Group mean cardiac output fell by 21% after the first and by 24% after the second dose of buffer (Table 2). Estimated total peripheral resistance was 0.073 ± 0.013 × 10⁻³ kPa l⁻¹ s before haemorrhage, 0.082 ± 0.002 × 10⁻³ (P < 0.05) after the first and 0.086 ± 0.017 × 10⁻³ (P < 0.05) after the second buffer infusion.

The addition of meclofenamate (6 mg/kg) immediately after haemorrhage in group 2 made little additional difference to systemic haemodynamics. The small fall in arterial pressure seen in group 1 after haemorrhage was not attenuated by meclofenamate in group 2 and the reduction in mean cardiac output was almost identical (58 and 60 ml min⁻¹ kg⁻¹ in groups 1 and 2 respectively).

When captopril (1 mg/kg) was given after haemorrhage in group 3, the mean arterial pressure fell only slightly more than with buffer or meclofenamate. Similarly captopril had no effect on the haemorrhage-induced change in mean cardiac output.

In group 2 the infusion of captopril as the second drug (i.e. after meclofenamate) resulted in a fall in arterial pressure of only 3 mmHg. Cardiac output, however, increased (Table 2) and estimated total peripheral resistance fell from 0.096 ± 0.022 × 10⁻³ to 0.08 ± 0.028 × 10⁻³ kPa l⁻¹ s.

In group 3 infusion of meclofenamate only partially reversed the hypotensive effect of

### Table 1. Changes in circulating hormone concentrations before and after haemorrhage and after infusion of first drug

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<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<td></td>
<td>Pre-haemorrhage</td>
<td>Immediately after haemorrhage</td>
<td>After buffer 1</td>
</tr>
<tr>
<td>Plasma renin activity (ng of ANG I b⁻¹ ml⁻¹)</td>
<td>25.0 ± 5.2</td>
<td>78.3** ± 13.4</td>
<td>53.1 ± 11.0</td>
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<tr>
<td>Plasma noradrenaline (nmol)</td>
<td>0.46 ± 0.04</td>
<td>0.79*** ± 0.10</td>
<td>0.48 ± 0.08</td>
</tr>
<tr>
<td>Plasma adrenaline (nmol)</td>
<td>0.30 ± 0.05</td>
<td>0.87*** ± 0.15</td>
<td>0.21 ± 0.07</td>
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TABLE 2. Systemic and organ haemodynamics before haemorrhage and after each drug infusion
Mean values ± SEM are shown. Comparison of post-haemorrhage with pre-haemorrhage values by Student's paired t-test:
* P < 0.05; ** P < 0.01; *** P < 0.001.

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<th>Group 1</th>
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<tr>
<td><strong>Pre-haemorrhage</strong></td>
<td><strong>Haemorrhage</strong></td>
<td><strong>Pre-haemorrhage</strong></td>
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<td><strong>Mean blood pressure (mmHg)</strong></td>
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<tr>
<td>Before buffer 1</td>
<td>After buffer 2</td>
<td>Before meclofenamate</td>
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<td>87 ± 4</td>
<td>76 ± 6</td>
<td>79* ± 5</td>
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<td><strong>Cardiac output (ml min⁻¹ kg⁻¹)</strong></td>
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<td>272 ± 18</td>
<td>212** ± 17</td>
<td>208** ± 16</td>
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<td><strong>Right renal blood flow (ml min⁻¹ g⁻¹)</strong></td>
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<tr>
<td>8.82 ± 0.9</td>
<td>7.60 ± 1.2</td>
<td>8.13 ± 1.1</td>
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<td><strong>Splanic blood flow (ml min⁻¹ g⁻¹)</strong></td>
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<tr>
<td>6.35 ± 0.91</td>
<td>3.91 ± 0.76</td>
<td>4.97 ± 1.27</td>
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<tr>
<td><strong>Coronary blood flow (ml min⁻¹ g⁻¹)</strong></td>
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<tr>
<td>4.01 ± 0.49</td>
<td>4.44 ± 0.46</td>
<td>4.45 ± 0.57</td>
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Fig. 1. (a) Renal blood flow and (b) estimated renal vascular resistance before and after haemorrhage. Upper panels (group 1): left, before haemorrhage and, right, after each infusion of buffer (B) after haemorrhage. Middle panels (group 2): left, before haemorrhage and, right, after infusion of meclofenamate (M), and then captopril (C). Lower panels (group 3): left, before haemorrhage and, right, after infusion of captopril (C) and then meclofenamate (M). Comparison of pre-haemorrhage with post-haemorrhage values in each group: *P < 0.05; **P < 0.01; n = 7.
captopril, although the resulting mean arterial pressure (72 ± 14 mmHg) was not significantly different from that before haemorrhage (83 ± 8 mmHg). No further change in cardiac output occurred as the result of the meclofenamate infusion and estimated total peripheral resistance was only slightly elevated, from 0.073 ± 0.017 × 10⁻⁵ to 0.078 ± 0.016 × 10⁻⁵ kPa l⁻¹ s⁻¹.

**Effects of haemorrhage on organ blood flow**

Despite the reductions in arterial pressure and cardiac output after haemorrhage, in no case did organ blood flow fall significantly (Table 2).

**Renal changes after haemorrhage with buffer, meclofenamate or captopril**

Whereas after haemorrhage alone, mean renal blood flow was reduced by only 14% (P > 0.05), in the presence of meclofenamate mean flow fell by 31% (P < 0.005; Fig. 1; Table 2). Estimated renovascular resistance was unchanged after haemorrhage and buffer but increased by 62% after haemorrhage and meclofenamate (P < 0.05; Fig. 1).

After haemorrhage and captopril total renal blood flow remained unaltered. However, there was a significant decrease in estimated renovascular resistance after captopril (P < 0.05) (Table 2; Fig. 1).

**Renal effects after both meclofenamate and captopril had been infused after haemorrhage**

The renal response to the addition of captopril after meclofenamate was an abrupt fall in renal blood flow by 47% (Table 2; Fig. 1) and elevation of estimated renal vascular resistance by 87% (Fig. 1).

In group 3, despite the fact that the systemic changes recorded after infusion of the second drug (meclofenamate) were in the opposite direction to those observed in group 2, after infusion of the second drug (captopril) the effect on the renovascular bed was similar. Thus renal blood flow fell after meclofenamate by 45% (Table 2; Fig. 1) and renovascular resistance rose by 104% (Fig. 1).

**Haemodynamic effects in other organs**

Haemorrhage alone (group 1) reduced splenic blood flow in six of seven rabbits. The extent of the fall was not changed by the addition of meclofenamate (group 2), and was somewhat less after captopril (group 3) immediately after haemorrhage (Table 2). However, in control group 1 mean splenic blood flow had partially returned by the time of the second infusion of the buffer, and in groups 2 and 3 blood flow was further reduced by final infusions of captopril and meclofenamate respectively (Table 2). The resulting mean splenic vascular resistance was higher than pre-haemorrhage after the final drugs [captopril in group 2 (P < 0.02) and meclofenamate in group 3 (P < 0.05)], though not after buffer in group 1.

There was a tendency for increased coronary blood flow after haemorrhage and the first drug in all groups. Blood flow then remained constant in group 1, whereas the addition of either captopril or meclofenamate further increased coronary perfusion (Table 2). The resulting mean coronary vascular resistance was lower than pre-haemorrhage after the final drugs: captopril in group 2 (P < 0.01) and meclofenamate in group 3 (P < 0.01), though not after buffer in group 1.

There were no significant changes in blood flow to the brain or adrenal glands after haemorrhage or drug infusion.

**Discussion**

This study was designed primarily to investigate whether inhibitors of synthesis of either prostaglandins or angiotensin II modified the response of the kidney or other organs to mild haemorrhage in conscious rabbits. Thus the most important findings were the production of post-haemorrhagic renal oligaemia by meclofenamate, the prevention of the haemorrhage-induced renal vasoconstriction by captopril and the profound constriction of renal and splenic vessels when meclofenamate and captopril were combined after haemorrhage.

The systemic and regional vascular response to haemorrhage in experimental animals has been studied extensively [11-15]. The great majority of the published studies have, however, concentrated on the pathophysiological changes associated with hypovolaemic shock, a condition in which major changes occur in cardiovascular function at all levels from capillary permeability to cardiac performance. This was not the purpose of the present study, which was directed instead at using mild haemorrhage as a means by which vasopressor mechanisms could be activated.

In the present experiments a slow haemorrhage of 15% of total blood volume resulted in a 213% increase in plasma renin activity and 71 and 190% increases in plasma concentrations of noradrenaline and adrenaline respectively. Renal blood flow was maintained after haemorrhage
alone in the presence of a 13% reduction in arterial pressure and 21% reduction in cardiac output.

In a series of studies of 26% haemorrhage in conscious rabbits [13, 16, 17] 'de-efferented' and adrenalectomized rabbits responded by greater falls in arterial pressure and cardiac output than did intact animals, suggesting withdrawal of normal sympatho-adrenal vascular tone. Renal blood flow, however, was greater in the de-efferented than in normal animals after haemorrhage and renal vascular resistance fell. It was suggested that 'local dilator effects' [17] were activated in response to haemorrhage.

These and other studies leave two important questions unanswered. In response to mild blood loss what is the nature of the renal vascular dilator forces?; and when this reactive vasodilation is prevented does local or circulating angiotensin II contribute to renal vasoconstriction in the intact animal?

There is evidence that endogenous prostaglandins reduce renal vascular resistance after haemorrhage. Blood loss has been shown to increase prostaglandin E activity in canine renal venous blood [18] and renal medullary tissue [19], and prostacyclin and prostaglandin E dilate renal vessels in the rabbit [20].

When conscious dogs are subjected to mild and moderate haemorrhage the renal vasodilation is reversed by blockade of prostaglandin synthesis [14]. Renal vasodilation after haemorrhage in dogs is not prevented by phentolamine, propranolol, atropine or tripe lanamine, suggesting lack of participation by adrenergic, cholinergic or histaminergic receptors respectively. The present experiments provide strong support for participation by the prostaglandins in reducing vascular resistance in the kidney after haemorrhage. Renovascular resistance was unchanged after haemorrhage alone but increased substantially after administration of meclofenamate after haemorrhage, further supporting the view that in the kidney the 'local dilator effects' [17] in response to mild haemorrhage are at least partially mediated by reactive stimulation of dilator renal prostaglandins.

The second major problem addressed by the present experiments was identification of the contribution played by angiotensin II in the renal vasoconstrictor response to mild haemorrhage and unmasked by meclofenamate. Given just after haemorrhage, captopril completely prevented the haemorrhage-induced fall in renal blood flow (group 3), suggesting that, in the absence of converting enzyme inhibition, angiotensin II significantly contributes to renovascular constriction after blood loss. However, this interpretation has to be guarded as captopril, by acting as a kininase II inhibitor, may also interfere with breakdown of vasodilator bradykinin and may also have more non-specific vasodilator effects.

Haemorrhage stimulates the renin-angiotensin system and renal infusion of angiotensin II increases renal prostaglandin production [21]. Moreover there is certainly evidence that a decrease in renal blood flow can occur after haemorrhage independent of innervation. Aars & Akre [12] recorded renal vasoconstriction after section of the renal nerves, and in cross-circulation experiments showed an increase in renal vascular resistance when the kidney was perfused by blood from the rabbit subjected to haemorrhage.

After lesser degrees of blood loss non-neural factors may be acting together with increase in sympathetic nerve discharge in conscious animals. Results from the present experiments show clearly that the vasoconstriction induced by meclofenamate is not due to an unopposed action of angiotensin II when prostaglandin synthesis is suppressed, as when captopril was used to inhibit angiotensin converting enzyme substantial vasoconstriction still occurred after administration of meclofenamate. It would seem likely that after mild haemorrhage in conscious rabbits renal vasoconstriction after meclofenamate is mediated by sympathetic nerve activity. The mechanism is similar, therefore, to that after severe haemorrhage in conscious dogs when renal vasoconstriction after indomethacin is reduced by renal denervation [6].

Infusion of captopril in the third group of animals resulted in profound renal vasoconstriction rather than the renal vasodilation that might have been expected from the effects seen when captopril alone is given to unbled animals [2]. A similar phenomenon was seen in the spleen, in which severe vasoconstriction also occurred with the combination of haemorrhage, meclofenamate and captopril. One possible explanation for this renal and splenic vasoconstriction is as follows. Both haemorrhage and captopril will tend to activate the baroreflex by tending to cause a fall in blood pressure. Haemorrhage also increased circulating plasma adrenaline and noradrenaline levels. The result is an increase in prostaglandin release from the kidney and spleen, serving to maintain blood flow to these organs in the face of the increased pressor stimuli. Administration of meclofenamate in these circumstances, by inhibiting prostaglandin synthesis, leaves unopposed the increased α-adrenoceptor activation. If this ex-
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explanation is correct the intensity of the baroreflex-induced vasoconstriction would appear to be much greater in conscious rabbits than in anaesthetized dogs where infusion of a competitive angiotensin antagonist increased rather than decreased renal blood flow in the presence of haemorrhage and prostaglandin synthesis inhibition [6]. However, the explanation above is supported by observation in unbled animals, in which only the spleen showed profound vasoconstriction with the combination of meclofenamate and captopril, and the effect was prevented by the administration of phentolamine [2].

The possibility that there is some other explanation for this exaggerated renal vasoconstriction seen when captopril was combined with meclofenamate should be borne in mind. It may reflect increasing vasoconstrictor effect of meclofenamate with time. We believe this to be unlikely, however, as in the non-bled rabbit maximum renal vasoconstriction is seen 15 min after infusion of the drug, with a somewhat diminished effect by 30 min (L. J. Beilin, personal observations). This latter time interval would correspond closely to the final renal blood flow measurements in rabbits receiving meclofenamate followed by captopril in the present study (group 2).

In conclusion, these experiments lend support to the suggestion that local production of prostaglandins plays an important role in the maintenance of renal and splenic blood flow during mild haemorrhage. A combination of mild haemorrhage, meclofenamate and captopril resulted in considerable renal and splenic oligemia, effects we suggest that may result from enhanced and unopposed neurosympathetic vasoconstriction. The renal vasoconstriction induced by meclofenamate after haemorrhage is not mediated by angiotensin II. The observations described may have clinical implications for patients receiving drugs that inhibit prostaglandin synthesis combined with a converting enzyme inhibitor, particularly when the renal circulation is impaired by hypovolaemia, renal artery constriction, anaesthesia or surgery.

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