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Characterisation of functional endothelin receptors in the canine isolated perfused spleen

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Abstract

The endothelin receptor subtypes involved in the vasoconstriction, capsular smooth muscle contraction, prostaglandin E_2 and prostacyclin release induced by endothelin-1 have been investigated in the canine isolated perfused spleen using both the endothelin ET_A receptor antagonist FR 139317 and the endothelin ET_B receptor agonist IRL 1620. The isolated canine spleen was perfused with warmed (37°C) and oxygenated (95% O_2/5% CO_2) Krebs solution at constant flow with continuous recording of splenic arterial perfusion pressure and spleen weight. Samples of splenic venous effluent were collected to determine the amounts of prostaglandin E_2 and prostacyclin, measured by radioimmunoassay. Endothelin-1 (4–200 pmol) and IRL 1620 (20–1000 pmol) dose-dependently increased splenic arterial perfusion pressure but the former was more potent on a molar basis (the molar dose ratio IRL/endothelin-1 required to increase splenic arterial vascular resistance by 25% was approximately 33). The infusion of the nitric oxide inibitor N^{ω}-nitro-L-arginine methyl ester (10 μM), but not of the enantiomer N^{ω}-nitro-D-arginine methyl ester, significantly potentiated the increase in splenic arterial vascular resistance induced by endothelin-1. The infusion of FR 139317 (1 μM) markedly attenuated the increased splenic arterial perfusion pressure induced by endothelin-1 without affecting that evoked by IRL 1620. At the highest dose (200 pmol), endothelin-1 induced a small but significant capsule contraction as reflected by the reduction in the spleen weight. The infusion of FR 139317 (1 μM) abolished this contractile effect. IRL 1620 (in doses up to 1000 pmol) did not significantly affect the capsule tone. The administration of either endothelin-1 (20–200 pmol) or IRL 1620 (20-1000 pmol) caused the release of 6-oxo-prostaglandin $F_{1\alpha}$ (breakdown product of prostacyclin) and prostaglandin E_2 into the splenic venous effluent. The amount of both prostanoids released by endothelin-1 was significantly greater than that induced by IRL 1620. FR 139317 (1 μM) significantly reduced ($P < 0.05$) the release of both 6-oxo-prostaglandin $F_{1\alpha}$ and prostaglandin E_2 by endothelin-1 without affecting that released by IRL 1620. The results demonstrate that the release of prostaglandins and nitric oxide modulates the vasoconstrictor activity of endothelin-1 in the splenic circulation. Furthermore, the vasoconstriction and eicosanoids (prostacyclin and prostaglandin E_2) release by endothelin-1 are due to activation of both endothelin ET_A and ET_B receptors, although the former seems to be the predominant form. The splenic capsule contraction is mediated by activation of endothelin ET_A receptors only.

Keywords: Endothelin-1; IRL 1620; FR 139317; Prostacyclin; Nitric oxide (NO); Prostaglandin E_2; Endothelin receptor

1. Introduction

The 21-amino acid peptide endothelin-1 is a potent vasoconstrictor agent first isolated from the supernatant of cultured porcine aortic endothelial cells (Yanagisawa et al., 1988). The vasoconstrictor effects induced by endothelin-1 both in vivo (De Nucci et al., 1988a) and in certain microvascular beds in vitro such as rat mesentery (Warner et al., 1989), dog liver (Withrington et al., 1989), dog spleen (Grassi-Kassisse et al., 1994a), rabbit kidney and spleen (Rae et al., 1989) are accompanied by the release of potent vasodilator autacoids including nitric oxide, prostacyclin

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and prostaglandin E_2 which modulate the direct vasoconstrictor activity of endothelin-1.

Endothelin exerts its vasoconstrictor and indirect vasodilator actions through the activation of at least two distinct receptor types: ET_A (Arai et al., 1990) and ET_B (Sakurai et al., 1990). The direct vasoconstrictor action of endothelin-1 is mediated by activation, in different tissues, of either ET_A or ET_B receptors (Sumner et al., 1992; Okamura et al., 1992; Moreland et al., 1992; Godfraind, 1993; Warner et al., 1993); the indirect vasodilator effect is thought to result, principally, from activation of endothelin ET_B receptors (Clozel et al., 1992; Warner et al., 1993).

The aim of the present study was to further characterise the endothelin receptors in the canine isolated perfused spleen, with regard to the splenic vasoconstriction and capsular contraction (Withrington et al., 1992) and the release of both prostacyclin and prostaglandin E_2 (Grassi-Kassisse et al., 1994a). This was achieved by using both the endothelin ET_A receptor antagonist FR 139317 (Sogabe et al., 1993) and the endothelin ET_B receptor agonist IRL 1620 (Takai et al., 1992).

2. Material and methods

2.1. Canine perfused spleen

Mongrel dogs (mean weight 8.1 ± 0.5 kg, both sexes; provided by CEMIB-UNICAMP) were anaesthetised with pentobarbital sodium (Sagatal, 30 mg/kg). The surgical procedures involved in the isolation of the spleen have been described in detail previously (Corder et al., 1987). Briefly, following the isolation of the spleen from the connections with the stomach, pancreas and omentum, both the splenic artery and vein were separated in situ and the spleen removed. Heparin (5000 U) was administered 5 min before the spleen removal. The splenic artery and vein were cannulated and the spleen perfused via the arterial system with warmed (37°C) and oxygenated (95% O_2 + 5% CO_2) Krebs solution (pH 7.4). The arterial inflow was adjusted, by means of a perfusion pump, to provide a perfusion pressure of 20–40 mm Hg and once this flow was established it remained constant throughout the experiment. Splenic arterial perfusion pressure was measured with a strain gauge transducer (model PRC 21/3, Ugo Basile) incorporated into the splenic arterial line. Splenic arterial vascular resistance was calculated as mean splenic arterial perfusion pressure/mean splenic arterial flow. Changes in spleen volume were assessed indirectly by weighing the spleen continuously on a digital balance with a visual reading at frequent set intervals (Boatman and Brody, 1964). At the end of the experiment the spleen was cleared of surrounding tissues and weighed.

Endothelin-1, IRL 1620 and adrenaline were administered as single bolus injection (1 ml) through the splenic artery and were washed in immediately with 0.5 ml of Krebs solution to provide a total constant injectate of 1.5 ml. A standard dose of adrenaline (54 nmol) was used since previous experiments in identical preparations (Grassi-Kassisse et al., 1994a,b) showed that this dose occupied the mid position in the dose-response curve. The endothelin-1 receptor antagonist FR 139317 (1 μM) was infused through the arterial line at a flow rate of 0.1 ml/min. In the experiments designed to investigate the effect of FR 139317 on the responses induced by endothelin-1, IRL 1620 and adrenaline, these agonists were injected 15 min after the start of the FR 139317 infusion.

2.2. Radioimmunoassay (RIA) of prostaglandin E_2 and 6-oxo-prostaglandin $F_{1\alpha}$

Fifteen second-samples of the splenic venous effluent were collected before and during 90 s intervals for up to 7.5 min after intra-arterial injection of endothelin-1, IRL 1620 or adrenaline. The levels of prostaglandin E_2 and 6-oxo-prostaglandin $F_{1\alpha}$ (breakdown product of prostacyclin) were determined by direct RIA without prior extraction. The RIA procedure and the specificity of the antisera employed have been previously described (Salmon, 1978; Bakhle et al., 1985).

2.3. Experimental design

The experimental protocol consisted of the bolus injections of adrenaline (54 nmol) followed by a dose-response curve to endothelin-1 (20, 40 and 200 pmol); 15 min after starting the infusion of FR 139317 (1 μM), adrenaline and endothelin-1 were injected again. A similar protocol was applied to investigate the effects of IRL 1620.

2.4. Drugs

(±)-Adrenaline, N^{ω}-nitro-L-arginine methyl ester, 6-oxo-prostaglandin $F_{1\alpha}$ and endothelin-1 were obtained from Sigma (St. Louis, USA). N^{ω}-Nitro-D-arginine methyl ester was obtained from Bachem (Switzerland). 6-Oxo-5,6,8,9,11,14,15(*n*)-[^{3}H]prostaglandin $F_{1\alpha}$ (specific activity 150 Ci/mmol) was purchased from Amersham (Bucks., UK). Antiserum for 6-oxo-prostaglandin $F_{1\alpha}$ was a gift from J. Salmon (Wellcome Laboratories, Kent, UK). Sagatal and heparin were purchased from May and Baker (Dagenham, UK) and Roche (São Paulo, Brazil), respectively. FR 139317 ((*R*)2-[(*R*)-2-[[1-(hexahydro-1*H*-azepinyl]carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)]propyonyl] amino-3-(2-pyridyl) propionic

acid) was synthesised by Abbott Laboratories (Chicago, USA). IRL 1620 (Suc-[Glu^{9},Ala^{11,15}]endothelin-1-(8–21) was supplied by Dr. M. Takai (Ciba-Geigy, Japan).

Endothelin-1, IRL 1620 and adrenaline were diluted in Krebs solution prior to intra-arterial injection. FR 139317 (stock solution of 150 μM) was dissolved in 1 N NaOH and the pH corrected to 7.4.

2.5. Statistical analysis

The results are expressed as mean ± S.E.M. of n experiments. Significance of the data was calculated using Student's t-test and $P < 0.05$ was taken as significant.

3. Results

The mean spleen weight was 63.5 ± 8.0 g representing 0.75 ± 0.06% of body weight ($n = 22$). The mean splenic arterial flow was 20.7 ± 1.5 ml/min giving an initial mean splenic arterial vascular resistance of 1.65 ± 0.17 mm Hg (ml/min).

3.1. Changes in splenic arterial vascular resistance induced by endothelin-1, IRL 1620 and adrenaline

Intra-arterial bolus injections of endothelin-1 (4–200 pmol, $n = 4–9$) caused a long-lasting and dose-dependent increase in the splenic arterial perfusion pressure (Fig. 1 and Fig. 2). In contrast, bolus injections of IRL 1620 (20–1000 pmol, $n = 10$) caused small increases in splenic arterial vascular resistance of short duration. These vasoconstrictor responses to IRL 1620 were, at all dose levels, significantly greater than zero. The responses were graded with the doses since the response at 200 pmol was significantly greater than at 20 pmol ($P < 0.05$), 1 nmol was significantly greater than at 40 pmol ($P < 0.05$). In addition, the splenic vasoconstrictor responses to IRL 1620 were significantly smaller than those induced by equimolar doses of endothelin-1 (Fig. 2). For instance, the molar dose ratio IRL 1620/endothelin-1 required to increase splenic arterial vascular resistance by 25% was approximately 33.

Effect of the endothelin ET_A antagonist FR 139317

The intra-arterial infusion of FR 139317 (1 μM) did not affect basal splenic arterial perfusion pressure

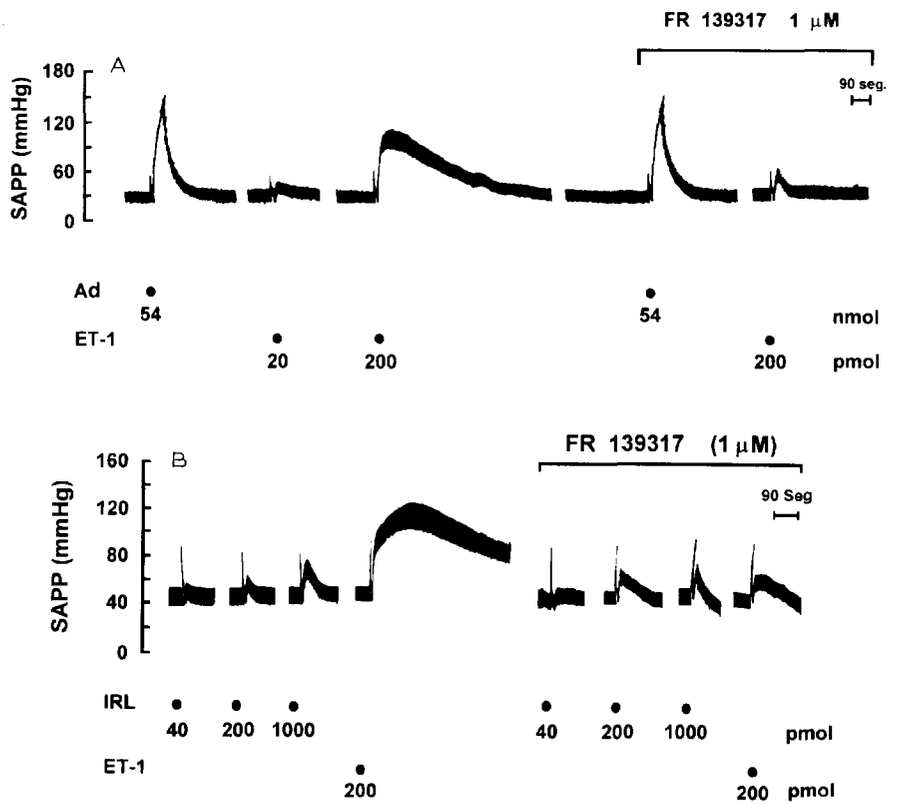

Fig. 1. Experimental recordings illustrating the increases in splenic arterial perfusion pressure (SAPP) in response to intra-arterial bolus injections of endothelin-1 (ET-1), an endothelin ET_B receptor agonist (IRL 1620) and adrenaline (Ad). Since the preparations were perfused at constant flow (panel A, 12 ml/min; panel B, 21 ml/min) these responses represent splenic vasoconstriction. During infusion of the endothelin ET_A receptor antagonist FR 139117 (1 μM) the vasoconstrictor responses to ET-1 (200 pmol) were markedly reduced whilst those to either Ad (panel A) or IRL 1620 (panel B) were not affected. Note that the y-axis scaling is different in panels A and B (spleen weight: A, 32 g; B, 48 g).

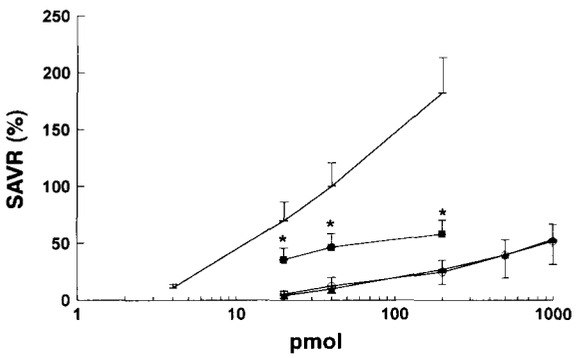

Fig. 2. Increase in splenic arterial vascular resistance (SAVR; expressed as percentage of basal value) caused by intra-arterial bolus injections of endothelin-1 (□) and the endothelin ET_B receptor agonist IRL 1620 (○) before (open symbols) and during (ET-1, ■; IRL 1620, ▲) the infusion of the endothelin ET_A receptor antagonist FR 139317 (1 μM). Individual points represent the means of 4–10 observations ± S.E.M. The splenic vasoconstrictor response to ET-1 is significantly reduced by endothelin ET_A receptor antagonism (* $P < 0.05$) whilst the responses to IRL 1620 are unchanged.

(34.2 ± 2.7 and 33.4 ± 2.8 mm Hg, before and during FR 139317 infusion, respectively, $n = 19$). However, at this concentration FR 139317 significantly reduced the increased splenic arterial vascular resistance induced by endothelin-1 without affecting that induced by either IRL 1620 (Fig. 1 and Fig. 2) or adrenaline (217.2 ± 31.4% and 224.1 ± 48.1, percent increase in splenic arterial vascular resistance before and during FR 139317 infusion respectively, $n = 18$).

Effect of cyclooxygenase and nitric oxide inhibition

The infusion of the cyclooxygenase inhibitor indomethacin (5.6 μM) significantly potentiated the splenic arterial vasoconstrictor response to both 1000 pmol IRL 1620 (14.4 ± 2.5% before and 111.7 ± 20.9% during indomethacin, $P < 0.05$, $n = 4$) and endothelin-1 (Grassi-Kassisse et al., 1994a). The infusion of the nitric oxide inhibitor N^{ω}-nitro-L-arginine methyl ester (10 μM) significantly potentiated the vasoconstrictor activity of endothelin-1 (19.7 ± 2.6, 41.6 ± 9.1 and 135.4 ± 16.4% increase in splenic arterial vascular resistance before and 61.0 ± 9.8, 123.6 ± 8.7 and 264.4 ± 5.3% increase in splenic arterial vascular resistance during N^{ω}-nitro-L-arginine methyl ester infusion, for 4, 20 and 40 pmol of endothelin-1, respectively, $n = 4$). The inactive enantiomer N^{ω}-nitro-D-arginine methyl ester (10 μM) had no effect on the vasoconstrictor responses to endothelin-1 (21.2 ± 1.6, 48.2 ± 10.7 and 152.2 ± 8.9% increase in splenic arterial vascular resistance during N^{ω}-nitro-D-arginine methyl ester infusion, for 4, 20 and 40 pmol of endothelin-1, respectively, $n = 4$).

3.2. Changes in capsular smooth muscle tone induced by endothelin-1, IRL 1620 and adrenaline

At the highest dose of endothelin-1 (200 pmol), the increase in splenic arterial vascular resistance was accompanied by a small, but significant, reduction in spleen weight (−3.2 ± 1.1 g, $P < 0.05$, $n = 9$), indicative of contraction of capsular smooth muscle. In contrast, IRL 1620 did not affect spleen weight in doses up to 1000 pmol ($n = 10$).

Effect of the endothelin ET_A antagonist FR 139317

The infusion of FR 139317 (1 μM) did not significantly affect basal capsule tone (spleen weight 171.0 ± 15.3 g before and 173.2 ± 15.0 g during FR 139317 infusion, respectively, $P > 0.4$, $n = 19$) but abolished the capsule contraction induced by endothelin-1 (200 pmol, $n = 9$, $P < 0.01$). In contrast, the capsule contraction induced by adrenaline (54 nmol) was not significantly affected by the concomitant infusion of FR 139317 (reduction in the spleen weight 28.0 ± 5.1 g before and 21.8 ± 3.2 g during FR 139317 infusion, $P > 0.2$, $n = 18$).

3.3. Release of 6-oxo-prostaglandin $F_{1\alpha}$ and prostaglandin E_2 induced by endothelin-1, IRL 1620 and adrenaline

Intra-arterial bolus injection of endothelin-1 (20–200 pmol, $n = 4$–9) induced a dose-dependent release of prostacyclin (6-oxo-prostaglandin $F_{1\alpha}$) into the splenic venous effluent (Fig. 3). The amount of 6-oxo-prostaglandin $F_{1\alpha}$ released by the highest dose of endothelin-1 was not significantly different ($P > 0.05$) from the standard dose of adrenaline (54 nmol; 10.4 ± 2.0 ng/ml, $n = 14$) but was significantly higher ($P < 0.05$) than that evoked by the highest dose (1000 pmol) of IRL 1620 (Fig. 3). The release of 6-oxo-prostaglandin $F_{1\alpha}$ induced by IRL 1620 was significantly different from

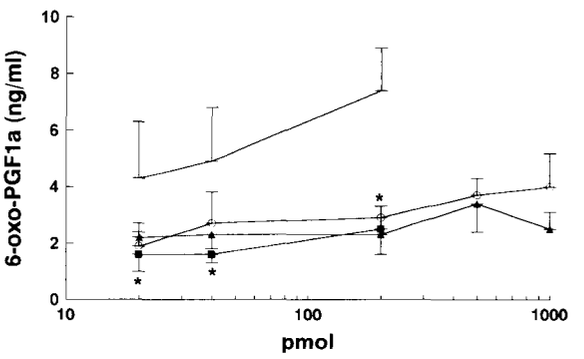

Fig. 3. Release of 6-oxo-prostaglandin $F_{1\alpha}$ (breakdown product of prostacyclin) into the splenic venous effluent following bolus injections of endothelin-1 (□) and the endothelin ET_B receptor agonist IRL 1620 (○) before (open symbols) and during (ET-1, ■; IRL 1620, ▲) the intra-arterial infusion of the endothelin ET_A receptor antagonist FR 139317 (1 μM). Points represent the means ± S.E.M. of 4–10 spleens. The release of 6-oxo-prostaglandin $F_{1\alpha}$ by ET-1 (20, 40 and 200 pmol) was significantly reduced by FR 139317 (* $P < 0.05$); in contrast there was no change in the release of 6-oxo-prostaglandin $F_{1\alpha}$ by IRL 1620. The *y*-axis represents the increase in 6-oxo-prostaglandin $F_{1\alpha}$ above basal release.

basal release (for 20 pmol, $P = 0.002$; for 1000 pmol, $P = 0.01$) and was dose-dependent since the release induced by 200, 500 and 1000 pmol was significantly greater ($P < 0.05$) than that induced by 20 pmol.

In addition, endothelin-1 released prostaglandin E_2 in a dose-dependent manner (10.0 ± 2.3, 15.6 ± 3.5 and 76.6 ± 31.7 ng/ml at 20, 40 and 200 pmol of endothelin-1, respectively). Although the endothelin ET_B receptor agonist IRL 1620 caused the release of prostaglandin E_2, the amounts even at the highest dose of agonist (1000 pmol) were significantly less (4.3 ± 0.9 ng/ml, $P < 0.05$) than that released by either the lowest dose of endothelin-1 (20 pmol; 10.0 ± 2.3 ng/ml; $n = 4$) or the standard dose of adrenaline (180.2 ± 47.4 ng/ml, $n = 8$).

Effect of the endothelin ET_A receptor antagonist FR 139317

The intra-arterial infusion of FR 139317 (1 μM) did not affect the basal release of 6-oxo-prostaglandin $F_{1\alpha}$ (1.8 ± 0.8 and 2.0 ± 1.1 ng/ml, before and during FR 139317 infusion respectively, $n = 19$, $P > 0.05$) but markedly reduced the release of 6-oxo-prostaglandin $F_{1\alpha}$ induced by endothelin-1 (Fig. 3). However, FR 139317 (1 μM) failed to affect the release of 6-oxo-prostaglandin $F_{1\alpha}$ induced by either IRL 1620 (Fig. 3) or adrenaline (54 nmol; 8.9 ± 1.3 ng/ml, $n = 10$).

The release of prostaglandin E_2 by the two highest doses of endothelin-1 was significantly reduced by FR 139317 infusion (40 pmol: 15.6 ± 3.5 ng/ml before and 8.1 ± 1.6 ng/ml during FR 139317 infusion; 100 pmol: 76.6 ± 31.7 ng/ml before and 11.4 ± 3.7 ng/ml during FR 139317). In contrast, the release of prostaglandin E_2 induced by both adrenaline (180.2 ± 47.4 ng/ml before and 123.5 ± 39.1 ng/ml during FR 139317) and IRL 1620 (4.3 ± 0.9 ng/ml before and 3.6 ± 0.7 ng/ml during FR 139317) was not significantly altered by endothelin ET_A receptor antagonism.

4. Discussion

The concept that endothelin ET_A receptors exist solely on vascular smooth muscle to induce vasoconstriction and that, in contrast, endothelin ET_B receptors on endothelial cells cause vasodilatation through the release of vasodilator autacoids (prostacyclin and NO) is now realised to be an oversimplified proposition. Recently, Gardiner et al. (1994) have reported that, in conscious rats, the vasoconstrictor effects of endothelin-1 and the selective endothelin ET_B receptor agonist, [$Ala^{11,15}$]Ac-endothelin-1-(6–21)] involved activation of endothelin ET_A, ET_B or both receptors depending on the agonist dose and vascular territory investigated. The present experiments in the canine isolated perfused spleen demonstrated that the pressor responses to endothelin-1 were selectively antagonised by FR 139317 (an endothelin ET_A receptor antagonist) and that an endothelin ET_B receptor agonist (IRL 1620) caused splenic vasoconstriction, although its activity was significantly less than that of endothelin-1. These results clearly demonstrate a predominant distribution of endothelin ET_A receptors in splenic vascular smooth muscle and, in addition, a relatively sparse distribution of endothelin ET_B receptors at the same site; both receptors, ET_A and ET_B, mediate vasoconstriction. We can offer no evidence for the further compartmentalization of endothelin receptors within the splenic microcirculation as demonstrated by Moreland et al. (1994) who, using a different vascular smooth muscle preparation, showed endothelin ET_A receptors at a high pressure arterial site with endothelin ET_B receptors differentially located in the low pressure site. However, in the present spleen preparation perfused with Krebs at a relatively low arterial pressure, the activation of endothelin ET_B receptors located on venous smooth muscle, leading to splenic venoconstriction, could increase the overall vascular resistance as observed with the endothelin ET_B agonist IRL 1620.

In addition, our results show that prostacyclin and prostaglandin E_2 release induced by endothelin-1 in the dog isolated perfused spleen is mediated by activation of both endothelin ET_A and ET_B receptors, although the former seems to be more important for endothelin-1 action. Although activation of endothelin ET_B receptors induces release of prostaglandins in some tissues (Matsuda et al., 1993; McMurdo et al., 1993; Kohan et al., 1993; Herman et al., 1993), there is evidence that the endothelin ET_A receptor is also implicated (Télémaque et al., 1993), an effect independent of vasoconstriction (Stanimirovic et al., 1993). As previously reported with endothelin-1 (Grassi-Kassisse et al., 1994a), prostaglandin release induced by IRL 1620 modulates its vasoconstrictor activity. We have now extended our observation to show that endothelin-1 also releases nitric oxide which in turn attenuates the vasoconstrictor effect of this peptide. This finding reinforces the physiological importance of the coupled release of prostaglandin and NO (De Nucci et al., 1988b; Hyslop and De Nucci, 1991) in the modulation of the vascular tone.

The endothelin ET_B receptor agonist IRL 1620 had no agonist activity on the canine capsule smooth muscle indicating the lack of functional endothelin ET_B receptors on the capsule. This is further supported by the finding that the endothelin ET_A receptor antagonist FR 139317 abolished endothelin-1-induced capsule contraction and that endothelin-1 is more potent than endothelin-3 in causing capsular contraction in the isolated blood perfused spleen of the dog (Withrington et al., 1992). Displacement studies using [^{125}I]endothelin-1 and [^{125}I]endothelin-3 showed higher affinity

for endothelin-1 than endothelin-3 in the pig (Hemsen et al., 1991) and rat (Jeng et al., 1990) spleen, indicating the predominance of endothelin ET_A receptors in splenic smooth muscle.

Endothelin-1 administration causes haemoconcentration in mice (Okumura et al., 1993), sheep (May et al., 1993) and rats (King et al., 1990; Valentin et al., 1991a; Sirois et al., 1992), but this increase in the haematocrit is probably due to plasma loss to the interstitial space rather than splenic contraction, since it also happens in splenectomised rats (Valentin et al., 1991b) and is associated with a marked reduction in whole-body plasma volume (Zimmerman et al., 1992). Our results demonstrating that endothelin-1 causes capsular contractions only at high doses support the interpretation that this organ does not contribute to the haemoconcentration observed following endothelin-1 administration.

The present experiments enlarge our previous observations on the endothelin receptor mechanisms involved in the complex changes in the control of the resistance and capacitance aspects of splenic circulation. They suggest that the local release of endothelin-1 will provoke marked changes in the distribution of blood within the spleen by activation of endothelin ET_A receptors causing intrasplenic vasoconstriction modified by local release of vasodilator prostanoids; the overall change in the splenic microcirculation may be determined by the distribution of the cell types and their spectrum of endothelin receptors. Relatively small changes in splenic capacity due to capsular and trabecular contraction through endothelin ET_A receptor activation may, in addition, alter the storage of cell types within the splenic compartments.

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