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Prostacyclin mediates antiaggregatory and hypotensive actions of endothelin in anaesthetized beagle dogs

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The effects of endothelin on blood pressure and *in vivo* aggregation of platelets were studied in anaesthetized beagle dogs. Intravenous administration of endothelin (0.03–0.3 nmol kg^{-1}) resulted in a dose-dependent transient hypotension followed by a long-lasting hypertension and inhibition of platelet aggregation. These changes were accompanied by dose-dependent elevation of plasma 6-keto prostaglandin $F_{1\alpha}$ levels. Pretreatment of the animals with acetylsalicylic acid significantly attenuated both the vascular and antiaggregatory responses to endothelin. These data provide evidence for *in vivo* release of prostacyclin by endothelin in anaesthetized dogs.

Introduction Endothelin isolated from porcine aortic endothelial cells (Yanagisawa *et al.*, 1988) has been demonstrated to be a potent constrictor of vascular smooth muscle in a variety of species *in vivo* and *in vitro* (Yanagisawa *et al.*, 1988; DeNucci *et al.*, 1988; Goetz *et al.*, 1988; Miller *et al.*, 1989). Endothelial cells, on the other hand, release both prostacyclin (Moncada *et al.*, 1976) and endothelium-derived relaxing factor (Furchgott & Zawadzki, 1980), which induce relaxation of the vascular smooth muscle. Thus, the production by the endothelium of substances with opposing vascular effects suggest it has a complex role in the regulation of vascular tone. In addition, endothelin inhibits *ex vivo* platelet aggregation in rabbits and this antiaggregatory effect of endothelin is blocked by indomethacin suggesting the involvement of prostanoids (Thiemermann *et al.*, 1988). In the present paper we present evidence that the *in vivo* antiaggregatory and hypotensive actions of endothelin are mediated by prostacyclin release.

Methods Experiments were performed on anaesthetized (sodium pentobarbitone, 30 mg kg^{-1}, i.v.) beagle dogs of either sex. Preparation of the animals has been described in detail elsewhere (Hermán *et al.*, 1986). Briefly, catheters were inserted into the left femoral artery and vein for monitoring mean arterial blood pressure and blood collection, respectively. An arteriovenous bypass was formed between the right femoral artery and vein. The arterial blood was directed through an extracorporeal filter with 30 μm pores by a roller pump. A few minutes after pumping the blood through the filter the perfusion pressure, measured proximal to the filter, started to increase linearly as a result of spontaneous platelet aggregation (Hermán *et al.*, 1986). Mean arterial blood pressure and filter pressure were monitored continuously by electromanometers (EM 31, Medicor, Budapest, Hungary) using Statham P 50 and P 23dB strain gauges (Hato Rey, Puerto Rico), respectively. After control measurements, porcine endothelin (Peninsula, 0.03–0.3 nmol kg^{-1} body wt) was given intravenously as a bolus. Four minutes after administration of endothelin 5 ml of blood was collected into plastic tube containing indomethacin (final concentration 10 μM).

Blood was mixed with [^{3}H]-prostaglandin $F_{1\alpha}$ ([^{3}H]-$PGF_{1\alpha}$) (4000 d.p.m.) for monitoring procedural losses and then prostanoids were extracted according to the method of Green *et al.* (1978). 6-keto-$PGF_{1\alpha}$ levels were measured by radioimmunoassay (IZINTA, Hungary). The antiserum had less than 0.9% cross reactivity with other prostanoids. Intraassay coefficient of variation was 4.6%. Values were corrected for individual recovery.

Results are expressed as means ± s.e.mean. Statistical analysis of the data was performed by one way analysis of variance using ranks (Friedman's test) followed by Wilcoxon–Wilcox's test to identify differences between groups (i.e. control versus different doses of endothelin).

Results Intravenous administration of endothelin resulted in a dose-dependent transient hypotension followed by long-lasting elevation in mean arterial blood pressure. The maximum falls in mean arterial blood pressure were 2 ± 1, 21 ± 15 and 29 ± 13 mmHg ($n = 4$) corresponding to 0.03, 0.1

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Table 1 Effects of endothelin on mean arterial blood pressure (MABP), filter occlusion rate (FOR), filter pressure (FP) and venous 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) concentrations in anaesthetized beagle dogs

	n	*For* (mmHg s^{-1})	*Decrease in FP* (mmHg)	*MABP* (mmHg)	*6-keto $PGF_{1\alpha}$* (ng ml^{-1} plasma)
Vehicle	6	—	—	—	0.68 ± 0.14
ET, 0.03 nmol kg^{-1}	4	0.25 ± 0.04	0	2 ± 1	0.60 ± 0.10
ET, 0.1 nmol kg^{-1}	4	0.70 ± 0.1*	9.5 ± 4*	21 ± 15*	1.04 ± 0.25
ET, 0.3 nmol kg^{-1}	6	1.41 ± 0.1*	34.1 ± 13*	29 ± 13*	1.35 ± 0.16*
ASA plus ET, 0.3 nmol kg^{-1}	4	0.12 ± 0.03§	0§	3 ± 1§	0.23 ± 0.15§

Animals were prepared as described under Methods. Four min after intravenous injection of various doses of endothelin (ET) blood was collected for determination of plasma 6-keto $PGF_{1\alpha}$ levels. Acetylsalicylic acid (ASA) was administered i.v. 30 min prior to injection of endothelin. Values are means ± s.e.mean. *n*, number of independent experiments. *$P < 0.05$ (compared to vehicle), §$P < 0.05$ (compared to ET, 0.3 nmol kg^{-1}).

and 0.3 nmol kg^{-1} of endothelin and the hypotensive effect lasted for 5–15, 10–90 and 40–180 s, respectively. These changes were accompanied by changes in filter pressure. The lowest dose of endothelin decreased the slope of the elevation of filter pressure on average for 52 s, 0.1 nmol kg^{-1} of endothelin stabilized filter pressure for 96 s, whereas the highest dose of endothelin employed reversed the spontaneous increase in filter pressure (average decrease was 19 mmHg), suggesting a disaggregatory action. This effect lasted on average for 182 s. In addition, endothelin enhanced venous 6-keto-$PGF_{1\alpha}$ concentrations in a dose-dependent fashion (Table 1).

When administration of endothelin (0.3 nmol kg^{-1}) was repeated in dogs pretreated with acetylsalicylic acid (Aspisol, Bayer, FRG, 25 mg kg^{-1}, i.v. 30 min before administration of endothelin) neither hypotension nor alteration of time course of changes in filter pressure were observed (Table 1).

Discussion The present study shows that in addition to its well established vasoconstrictor property, endothelin can exert hypotensive and *in vivo* antiaggregatory effects in anaesthetized beagle dogs. Several lines of evidence suggest that these actions of endothelin can be attributed to enhanced prostacyclin formation rather than to its direct effect. First, the hypotensive and antiaggregatory actions of endothelin were accompanied by a parallel rise in plasma 6-keto-$PGF_{1\alpha}$ levels and endothelin has been reported to release prostacyclin from guinea-pig and rat isolated lungs (DeNucci *et al.*, 1988). Secondly, acetylsalicylic acid at a dose that blocked prostacyclin formation, significantly attenuated the hypotensive and completely prevented the antiaggregatory responses to endothelin. Whether the slight decrease in mean arterial pressure after injection of endothelin in acetylsalicylic acid-pretreated animals was due to release of endothelium-derived relaxing factor is not known. Thirdly, the concentrations of prostacyclin measured in the present experiments were similar to those seen after administration of exogenous prostacyclin necessary to stabilize and/or reverse the spontaneous increase in filter pressure (Hermán *et al.*, 1986). Fourthly, endothelin (up to a concentration of 250 nM) added *in vitro* to platelet-rich plasma prepared from beagle dogs failed to modify the aggregatory response to ADP, platelet-activating factor or arachidonic acid (Filep *et al.*, unpublished observations). Fifthly, endothelin activates phospholipase A_2 with subsequent metabolism of arachidonic acid (Resink *et al.*, 1989).

In conclusion, the present findings provide direct evidence for *in vivo* release of prostacyclin by endothelin and strongly suggest that endothelin-induced immediate hypotension and antiaggregatory effects may be mediated through release of prostacyclin in anaesthetized beagle dogs.

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