Br. J. Pharmacol. (1989), **97**, 639–641

Endothelium-derived relaxing factor is an endogenous vasodilator in man

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Removal of venous endothelium in man leads to vasoconstriction and loss of dilator response to acetylcholine but not to glyceryl trinitrate. This pattern of responses can be accounted for by loss of endothelium-derived relaxing factor (EDRF), but not by loss of prostacyclin. This provides the first direct evidence for endothelium-dependent dilatation in man *in vivo*, and suggests that basal release of EDRF is a determinant of resting venous tone.

Introduction Vascular endothelium synthesises and releases a variety of vasodilator (Moncada *et al.*, 1977; Furchgott & Zawadski, 1980) and vasoconstrictor (Yanagisawa *et al.*, 1988) mediators. In animals the balance of effects favours vasodilatation since removal of endothelium results in vasconstriction (O'Neill, 1947; Lam *et al.*, 1988). In this study we have investigated whether this might also be so in human vasculature *in vivo*.

Methods Studies were performed on the large veins on the back of the hand. These vessels have no resting tone and with the subject relaxed, supine and in a warm environment, their distensibility (tone) remains constant (Nachev *et al.*, 1971). The study, which had local Ethical Committee approval, was performed on 22 healthy volunteers aged 18–32 years who gave their informed consent. Laboratory temperature was kept constant between 26–28°C. Venous diameter was recorded by measuring the linear displacement of a light-weight probe resting on the skin over the summit of the vein when the pressure in a congesting cuff placed around the upper arm was lowered from 40 mmHg to 0 mmHg (Nachev *et al.*, 1971). Drugs, or physiological saline, were infused continuously at 0.25 ml min^{-1} through a 23 SWG needle placed 10–15 mm upstream from the point of measurement.

Endothelium was removed by irrigating a 3–4 cm segment of the vein with distilled water (Bolton *et al.*, 1984). The segment was temporarily isolated by means of occluding wedges. An 'infusion' needle was placed at one end of the segment, a 'withdrawal' needle at the other, and the lumen irrigated with distilled water given at 5 ml min^{-1} for 30–40 min. After irrigation the wedges were removed and vein diameter measured. Venous responses to local infusions of noradrenaline (NA), acetylcholine (ACh) and glyceryl trinitrate (GTN) were measured before, and at 2 and 14 days after, irrigation. Subjects took soluble aspirin (600 mg) 30 min before the first control measurements were made and then daily for the next 7 days. Aspirin was again taken 30 min before recordings in those subjects restudied at 14 days.

Results are presented as mean ± s.e. mean and compared by use of Student's *t* test for paired data when $P < 0.05$ was considered significant. Drug-induced constriction is expressed as a percentage of vein diameter recorded during infusion of saline; dilatation is expressed as percentage increase in diameter from that of the NA-constricted vein.

Results In 16 subjects veins were irrigated with distilled water. Two of the irrigated segments thrombosed and could not be studied further. Both subjects had failed to comply with the study protocol by not taking aspirin.

In one set of experiments, involving 9 subjects, vein diameter was measured before, and 30 min after, irrigation. In each subject irrigation decreased resting vein diameter; pre-irrigation diameter was 1.31 ± 0.11 mm, post-irrigation diameter 0.60 ± 0.11 mm ($P < 0.01$). In 6 subjects measurements were repeated at 48 h, and in each the vein diameter remained reduced (0.69 ± 0.19 mm, $P < 0.01$; Figure 1). In 2 subjects, measurements were repeated at 14 days, and vein diameter had returned to the pre-irrigation size (Figure 1).

Evidence of smooth muscle integrity was sought by measuring venous responses to infusions of NA before, and 48 h after, irrigation in 6 subjects. NA (60 ± 13.9 pmol min^{-1}) caused a 51.6 ± 9.4% reduction in vein diameter before irrigation; after irrigation a similar reduction in diameter (55.6 ± 4.8%) was produced by NA (38 ± 13.7 pmol min^{-1}); the doses of NA were not significantly different. On both occasions the constriction was maintained throughout the infusion and

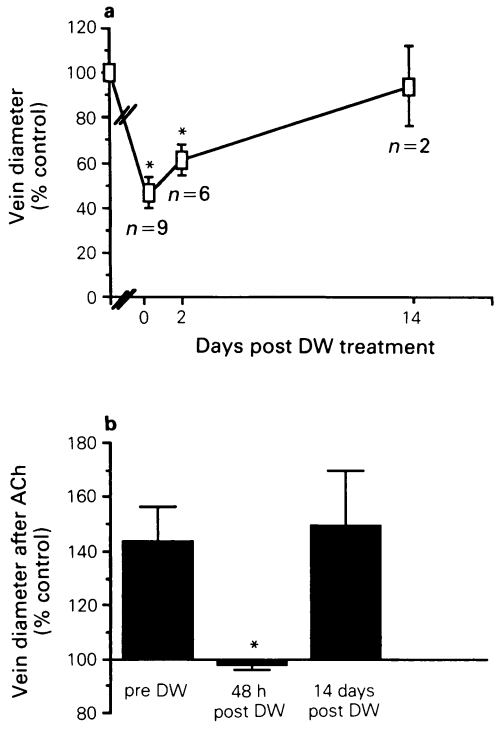

Figure 1 (a) Venoconstriction in man following removal of vascular endothelium *in vivo* with time. Vein diameter was measured at a distension pressure of 40 mmHg before (control), and at 30 min, 48 h and 14 days after, removal of endothelium with distilled water (DW) and is expressed as a percentage of the control; * denotes significantly different from control, $P < 0.01$. (b) The response of noradrenaline preconstricted dorsal hand vein to acetylcholine (1 nmol min^{-1}) before, 48 h and 14 days after removal of the endothelium by DW. Vein diameter is expressed as a percentage of the diameter recorded during infusion of NA alone; * denotes significantly different from the dilatation to ACh seen pre-DW, $P < 0.02$.

reversed at the same rate on stopping the drug. In these subjects pharmacological evidence for loss of endothelium was sought by use of ACh. In vessels preconstricted to a similar degree by NA, ACh (1 nmol min^{-1}) caused a 44 ± 13% dilatation before irrigation, but in no subject did this dose cause dilatation on the second day following irrigation ($P < 0.02$; see Figure 1). In 2 subjects studied at 14 days the dilator response to ACh was restored, and was identical to the pre-irrigation response (Figure 1).

In a second set of experiments the response of denuded vessels to GTN was examined. Five subjects were studied. In 4, denudation caused reduction of resting vein diameter (pre-irrigation control size 1.05 ± 0.27 mm; second day post-irrigation size 0.5 ± 0.08 mm). The fifth subject, however, failed to venoconstrict following denudation and showed no response to NA, even in doses of up to 800 pmol min^{-1}. It was assumed therefore, that in this subject the irrigation had impaired smooth muscle function. This subject was not studied further and was excluded from analysis. In the subjects in whom denudation caused a reduction in resting vein diameter, infusion of GTN into veins pre-constricted to a similar degree with NA, caused dose-dependent dilatation which was the same before, and after, denudation. Before denudation GTN, 3 and 6 pmol min^{-1}, caused the NA pre-constricted vein to dilate by 53.8 ± 9.9% and 77.3 ± 12.6% respectively, and two days after denudation by 53.8 ± 6.1% and 80.0 ± 10.6%. In all 4 subjects infusion of a larger dose of GTN (50 nmol min^{-1}) on the second day increased resting vein diameter from 0.50 ± 0.08 mm to 0.93 ± 0.21 mm ($P < 0.05$), returning it almost to the pre-irrigation control size (1.05 ± 0.27 mm).

In a third set of experiments, aspirin was infused into endothelium-intact veins of 6 subjects for 30 min, to give a calculated plasma concentration within the vein segment of 2 mM. Aspirin neither changed basal vessel tone (mean diameter before aspirin 1.2 ± 0.05 mm, after aspirin 1.23 ± 0.06 mm), nor altered the constrictor response to NA. The reduction in vein diameter produced by NA (80 pmol min^{-1}) was 49.6 ± 6.5% before aspirin, and 53.1 ± 5.4% after aspirin.

Discussion The results of this study provide the first direct evidence of endothelium-dependent dilatation in man *in vivo*, and demonstrate that the endothelium exerts a continuous dilator influence. Evidence for the presence of EDRF is based upon the observations that in NA pre-constricted dorsal hand veins with intact endothelium ACh and GTN dilate the vessels, whereas after irrigation with distilled water, only GTN is effective.

In addition we present evidence for basal EDRF release independent of ACh. Removal of the endothelium led to venoconstriction which gradually reversed over two weeks, a time by which the endothelial lining would have regenerated (Hayashi *et al.*, 1988) and the dilator response to acetylcholine had returned.

Basal release of prostacyclin (PGI_2) is unlikely to account for our findings as local infusion of aspirin altered neither resting vessel tone nor the constrictor response to NA.

Platelet-derived constrictor substances are also unlikely to contribute to the maintained constriction; first, the subjects took aspirin to inhibit

thromboxane synthesis; second, the constriction following denudation was the same at 30 min as at 48 h, yet platelet adhesion and activation vary with time, and would be greatest immediately after endothelial removal.

In conclusion, we suggest that EDRF plays a physiological role in the control of venous tone in man. Confirmation, however, must await a specific inhibitor of EDRF for use *in vivo*.

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(Received March 10, 1989
Accepted March 31, 1989)