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# INTRAVASCULAR BIG ENDOTHELIN INCREASES CIRCULATING LEVELS OF ENDOTHELIN-1 AND PROSTANOIDS IN THE RABBIT

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Human Big-Endothelin (Big-ET(h)) is the 38 amino acid precursor of Endothelin-1 (ET-1) which is formed by an atypical chymotrypsin-like cleavage between $Trp^{21}$ and $Val^{22}$ (1). Big-ET(h) has only 1/100 th of the potency of ET-1 on isolated tissues but as a pressor agent *in vivo* Big-ET(h) is equipotent to ET -1. We have now investigated the possible conversion of injected Big-ET(h) to ET-1 *in vivo* (2).

## MATERIAL AND METHODS

Adult, male rabbits (NZW, 2.5-3 kg) were anaesthetised with sodium pentobarbitone (20-30 mg/kg). A catheter was inserted into the left ventricle via the right carotid artery, and connected to a Grass Polygraph to measure left ventricular systolic pressure (LVSP). Big-ET(h) or ET-1 (Peptide Institute) was injected into the left ventricle. When required, indomethacin (5 mg/kg; Sigma) was injected via the left ear vein to prevent prostaglandin release (3).

Blood samples (2ml) were taken from the left ventricle 30 and 5 min before and 1, 5, 15, 30 and 60 min after bolus injections of Big ET(h) or ET-1 (1 and 3 nmol/kg). The blood samples were centrifuged (1400 *g* for 20 s) to obtain platelet-rich plasma (PRP) in order to measure *ex vivo* inhibition of ADP-induced platelet aggregation as previously described (4).

The samples were then further centrifuged (15000 *g* for 1 min) to obtain platelet-poor plasma (PPP). The samples (300 µl) were purified through Sep-Pak minicolumns (C-18), eluted with methanol (3 ml) and subsequently evaporated at 40 °C. The dessicated residue was redissolved in assay buffer (125 µl), prior to measurement of ET-1-like immunoreactivity. A double antibody assay for ET-1 or ET-2 (Amersham) with a modified anti-sera with 0.1% cross-reactivity for Big-ET (h) was used. The recovery of ET-1 through the separation procedure was higher than 85 % at the $IC_{50}$ of standard displacement curves in both assay buffer and PPP. All the results represent the mean ± S.E.M of at least 4 experiments. Significant differences were calculated by two way analysis of variance and/or paired t-test. P values $< 0.05$ were considered as significant.

## RESULTS AND DISCUSSION

Big-ET(h) and ET-1 (1 nmol/kg) were equipotent (peak increase of LVSP: 19+4 mmHg at 5 min and 20+4 mmHg at 1 min respectively) as pressor agents. Characteristically, as demonstrated in other species (2), Big-ET(h) did not induce an initial depressor effect as does ET-1 (3). However, the pressor response was of equal duration (30 min) for Big-ET and ET-1. As for ET-1 (4), indomethacin significantly potentiated the pressor response to Big-ET(h) (fig 1A). Bolus injection of Big-ET(h) caused the inhibition of *ex vivo* platelet aggregation (fig 2B) which was not seen in animals treated with indomethacin.

A bolus dose of Big-ET (h) (3 nmol/kg) increased eightfold (34 fmol/ml plasma) the amount of ET-1 detected in the circulation 1 minute after injection (fig 2A). At 5 min, there was a similar amount and even at 60 min, the plasma ET-1 level was still substantially above control (fig 2A). A similar time course was seen for Big-ET(h) at 1 nmol/kg (results not shown). In contrast, a significant increase of immunoreactive ET-1 following administration of ET-1 (1 nmol/kg) could be detected for only 5 min (fig 2B). This short life of ET-1 in the circulation is similar to that seen in other species (5) showing that the effect of ET-1 on blood pressure greatly outlasts its presence in the circulation. Thus, taking into account the lack of activity of Big-ET(h) on vascular

tissues *in vitro*, our results are best explained by a) a lack of removal of Big-ET(h) from the circulation coupled with b) a gradual conversion to the active ET-1 over 60 min, contributing to the long-lasting pressor effect. Interestingly, with Big-ET(h), the peak levels of ET-1 attained were lower than with ET-1, suggesting that the

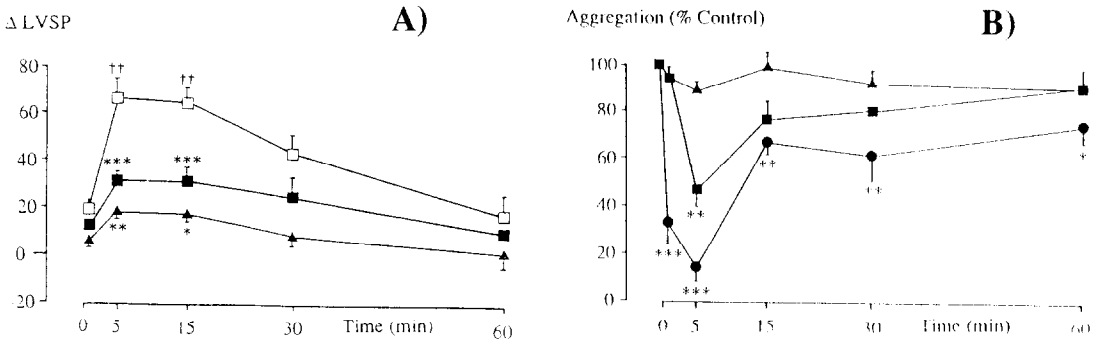


Fig 1A. Time course for changes in left ventricular systolic pressure after injection of Big-ET(h) at either 1 nmol/kg (▲) or 3 nmol/kg (■). Note that with Big-ET(h) (3 nmol/kg) after indomethacin treatment (□) there was a substantial potentiation of the pressor effect.
Fig 1B. Inhibition of platelet aggregation *ex vivo* in the same experiments as fig 1A. with the addition of ET-1 (1 nmol/kg (●) (*P<0.05; ††,**P<0.01; ***P<0.001).

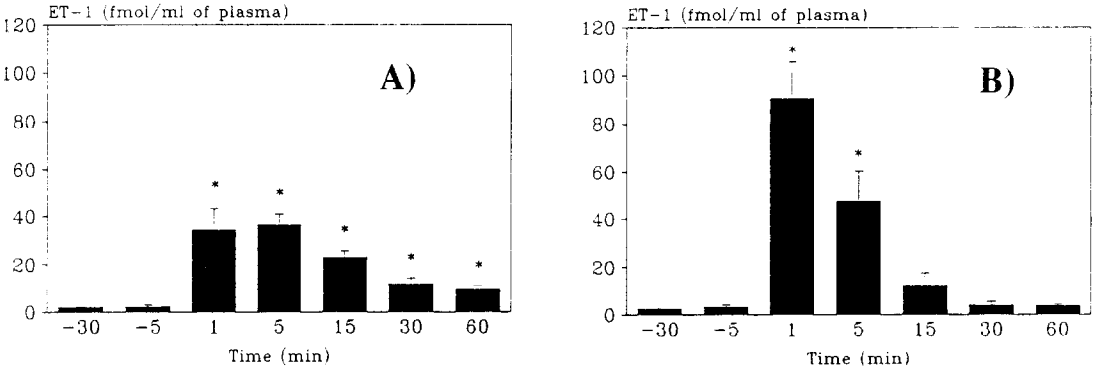


Fig 2. Plasma levels of ET-1 following bolus injections of Big-ET (h) (3 nmol/kg) (fig 2A) or ET-1 (1 nmol/kg) (fig 2B) (*P<0.05).

continued slow release of ET-1 from circulating Big-ET(h) helps to maintain the pressor effect. This is supported by comparing the peak blood pressure increase and radioimmunoassay results after Big-ET(h) and ET-1; with Big-ET(h) the peak occured at 5 min rather than at 1 min as with ET-1. For either ET-1 or Big-ET(h) to be active on vascular smooth muscle *in vivo*, they have to reach the muscle by penetrating the endothelium. It is probably during this process that prostacyclin release is induced. Thus we have no evidence for a different location from the endothelium for the converting enzyme for Big-ET(h).

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