Coronary vasoconstriction in the conscious rabbit following intravenous infusion of L-N\(^G\)-nitro-arginine


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Intravenous infusion of L-N\(^G\)-nitro-arginine, an inhibitor of endothelial nitric oxide (NO) synthesis, produced vasoconstriction in the coronary, cerebral, renal and duodenal vascular beds of the conscious rabbit. In this study, using radiolabelled microspheres, we provide in vivo evidence for a basal NO-dependent vasodilator tone in the coronary vascular bed.

Introduction

Intravenous (i.v.) infusion of L-N\(^G\)-monomethyl-arginine (L-NMMA), an inhibitor of endothelial nitric oxide (NO) synthesis, increases blood pressure (BP) in anaesthetized rabbits and produces vasoconstriction in a number of vascular beds in the conscious rat (Rees et al., 1989; Gardiner et al., 1990), implying a fundamental role for NO in the maintenance of normal resting vascular tone in vivo. Results from in vitro experiments suggest a similar role for NO in the coronary circulation (Amezcua et al., 1989). In the present study, by use of a radiolabelled microsphere technique in conscious rabbits, we now show that i.v. infusion of the NO-synthesis inhibitor L-N\(^G\)-nitro-arginine (L-NOARG, Moore et al., 1990) produces coronary vasoconstriction in vivo.

Methods

Male New Zealand White rabbits (2.5-3.5 kg, Foxfield) were anaesthetized with 0.9% alphaxalone/0.3% alphadalone (0.25 ml kg\(^{-1}\)) i.v. plus supplements, Saffan, Pitman-Moore) and cannulae were implanted for BP measurement and arterial blood reference sampling (right femoral artery), drug infusion (left jugular vein) and microsphere administration (left ventricle (LV) via left carotid artery). After a 24 h recovery period rabbits were placed in canvas restraining bags for the experimental procedure. BP and heart rate (HR) were recorded continuously and, following a 40 min stabilization period, baseline values were noted and the first suspension of microspheres (\(^{113}\)Ce) administered. Throughout the 75 s microsphere administration cycle a reference blood sample was withdrawn from the femoral artery at 2 ml min\(^{-1}\). Immediately prior to administration, approximately 300,000 microspheres (15 μm diameter, New England Nuclear) were suspended in 1 ml 0.02% Tween 80 by sonication and vortex mixing for 2 min. The microsphere suspension, followed by 1 ml heparinized saline (100 u ml\(^{-1}\)), was then steadily administered into the LV cannula over a period of 10 s starting 15 s into the cycle. The reference blood sample, empty aliquot vial and administration line were retained for counting.

The second microsphere suspension (\(^{103}\)Ru) was administered in the same manner after a 20 min i.v. infusion (0.2 ml min\(^{-1}\)) of either vehicle (saline) or L- or d-NOARG (0.5 mg kg\(^{-1}\) min\(^{-1}\)). Chemical Dynamics). Each animal received only one infusion and, following the second microsphere administration, was killed with an overdose of pentobarbitone. Tissues of interest were then removed and the left ventricular wall was sectioned into sub-epicardial, mid-myocardial and sub-endocardial layers (Hof, 1983). The initial microsphere suspensions and all samples were counted in a Packard 5530 gamma counter. Tissue blood flows (TBF) and cardiac output (CO), normalized for tissue and body weights, were derived by the reference flow principle (Heymann et al., 1977) following background and crosstalk corrections (Packard Compusphere). Tissue vascular and total peripheral resistances (TVR and TPR) were calculated as BP/TBF and BP/CO respectively. Between group comparisons of baseline values were made by analysis of variance (ANOVA) and the significance of drug-induced changes was tested by Student's paired t test. A P value < 0.05 was taken to indicate a significant difference.

Results

No between group differences in absolute pre-infusion values (Table 1) were observed with the exception of higher TVR (ileum and skin) and TPR in the saline group. The latter difference was only significant when compared to the d-NOARG group. Infusion of d-NOARG (0.5 mg kg\(^{-1}\) min\(^{-1}\)) produced a slight fall in CO but, in common with saline infusion, no change in BP, HR, or TPR and no vasoconstriction in any vascular bed studied. However, the same dose of L-NOARG produced significant increases in TVR in the vascular beds of the heart, kidneys, brain and duodenum. These effects, which were accompanied by increases in TPR and BP (31 and 10%) and falls in HR and CO (16 and 16%), are summarized in Figure 1. Coronary vasoconstriction occurred throughout the left ventricular wall and was statistically significant in the sub-endocardial and mid-myocardial layers. d-NOARG produced significant vasodilatation in the vascular bed supplying the back muscle.

Discussion

Our results confirm previous observations of renal, mesenteric and cerebral vasoconstriction following i.v.

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\begin{array}{c|c|c|c|c}
\text{Tissue} & \text{Brain} & \text{Heart} & \text{Epi} & \text{Mid} \\
\hline
\text{BP% change} & * & * & * & *
\end{array}
\]

\[
\begin{array}{c|c|c|c|c|c}
\text{Tissue} & \text{End} & \text{Kidneys} & \text{Back muscle} & \text{Leg muscle} & \text{Skin} \\
\hline
\text{BP% change} & * & * & * & * & *
\end{array}
\]

\[
\begin{array}{c|c|c|c|c|c}
\text{Tissue} & \text{Colon} & \text{Ileum} & \text{Duodenum} & \text{Stomach} \\
\hline
\text{BP% change} & * & * & * & *
\end{array}
\]

Figure 1 Effect of a 20 min i.v. infusion (0.2 ml min\(^{-1}\)) of vehicle (saline, open columns, n = 5), L-N\(^G\)-nitro-arginine (L-NOARG, 0.5 mg kg\(^{-1}\) min\(^{-1}\) solid columns, n = 6) or d-NOARG (0.5 mg kg\(^{-1}\) min\(^{-1}\)) hatched columns, n = 7) on tissue vascular resistance in conscious rabbits. Abbreviations as in Table 1. Columns show percentage changes from pre-infusion values calculated from mean data presented in Table 1. * P < 0.05 and ** P < 0.01 (paired Student's t test applied to absolute changes shown in Table 1).

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administration of an inhibitor of NO synthesis (Gardiner et al., 1990). More importantly, the present study demonstrates that acute i.v. infusion of such an agent results in coronary vasoconstriction in a conscious animal.

Inhibition of NO-dependent responses by arginine analogues is L-arginine specific and the in vitro effects of L-NOARG are reversed by L- but not D-arginine (Moore et al., 1990). This specificity is an established criterion for invoking inhibition of NO production as being responsible for the effects of arginine analogues. In the present study this requirement was met by the demonstration that only L-NOARG produced vasoconstriction.

D-NOARG produced significant vasodilation in the vasculature of the back muscle. Our results can give no indication of the mechanism for this effect but a positive interaction between NO and D-NMMA has recently been demonstrated in vitro (Boulangier et al., 1990).

Our study indicates that there is a basal NO-dependent vasodilator tone in a number of vascular beds, including the coronary circulation. This is likely to be of particular importance in the heart since oxygen extraction is already near maximum at rest and coronary vascular resistance is a major determinant of adequate oxygen delivery to the myocardium. The demonstration that inhibition of an endogenous NO synthesis results in coronary vasoconstriction in vivo supports the proposal (Vanhoutte & Houston, 1985) that pathophysiological loss of normal endothelial function may predispose to coronary vasospasm and ischaemia.

Note added in proof

Since submission of this manuscript our attention has been drawn to the work of Chu and coworkers (1990) who demonstrated coronary vasospasm in conscious dogs following infusion of L-NMMA directly into the left atrium of the heart.

References


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