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Vasoconstrictor and vasodilator effects in normal and atherosclerotic conscious rabbits

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1 Rabbits were fed a cholesterol-rich diet for 5 two-week intervals. Polyvinyl catheters were then implanted into the femoral artery and vein. Dose-response curves to acetylcholine (ACh), noradrenaline (NA), phenylephrine (Phen) and angiotensin II (AII), were obtained in 6 cholesterol-fed and 6 control rabbits before and after isradipine (code name PN200-110) $100\,\mu g\,kg^{-1}$. After these experiments the animals were killed and aortic rings were suspended in an organ bath. ACh but not nitroprusside-induced relaxation was impaired in atherosclerotic but not in control preparations.

2 ACh decreased blood pressure dose-dependently in both groups of rabbits even though ACh did not relax the aortae of the same rabbits *in vitro*.

3 Blood pressure effects reflect mostly changes in resistance vessels. The pressor effects of NA, Phen and AII were enhanced in atherosclerotic compared with normal rabbits.

4 After a dose of $100\,\mu g\,kg^{-1}$ isradipine the dose-response curves of all agents were shifted to the right. The differences between atherosclerotic and control rabbits disappeared, except for the AII-induced pressor response, which remained enhanced in atherosclerotic animals. The calcium antagonist thus only partly corrected the atherosclerosis-associated hyperresponsiveness to vasoconstrictor agents.

Introduction

Atherosclerosis changes the reactivity of many blood vessels. This has been shown both in experiments *in vitro* and *in vivo*. The enhancement of the vasoconstrictor effect of 5-hydroxytryptamine (5-HT) was among the first of such changes demonstrated experimentally (Henry & Yokoyama, 1980; Shimokawa *et al.*, 1983; Kawachi *et al.*, 1984). Acetylcholine (ACh)-induced relaxation is diminished in atherosclerotic vessels (Freiman *et al.*, 1986, Habib *et al.*, 1986; Bossaller *et al.*, 1987a) and this can also be demonstrated angiographically in patients (Ludmer *et al.*, 1986). The present experiments were designed to investigate the effects of ACh and several pressor agents in intact conscious rabbits. We hoped to find changes in responsiveness, which could be potentially useful in detecting and characterizing atherosclerosis *in vivo*.

Calcium antagonists modulate the constrictor effects of agents which increase calcium entry into smooth muscle cells (Cauvin *et al.*, 1983). Correction of altered responsiveness in atherosclerotic animals by a calcium antagonist would suggest that the defect is located in smooth muscle rather than endothelium (Jayakody *et al.*, 1987a,b). To our knowledge the effects of calcium antagonists have not been investigated in atherosclerotic animals *in vivo* and this was also a purpose of the present experiments.

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Methods

Conscious rabbits

Mongrel rabbits were obtained at 8–10 weeks of age and with a weight of about 1 kg. Half of the animals were fed the normal rabbit chow, half of them received the same chow with 2% cholesterol added for two weeks, followed by a 2 week period on normal chow. This was repeated 5 times, over a total of 20 weeks. This procedure results regularly in severe aortic atherosclerosis, while the animals stay healthy and gain weight normally.

The rabbits were prepared for the experiments as described in detail previously (Hof & Scholtysik, 1983). Briefly, the rabbits were acclimatised to the

laboratory for several days. Fine polyvinyl catheters were then inserted into the femoral artery and vein in an aseptic operation under pentobarbitone anaesthesia. These catheters were guided subcutaneously to the back. Before waking up, the animals were fitted with jackets, to protect the catheters. Five or more days after surgery the experiments were carried out with the animals sitting quietly in a cage narrow enough to prevent them from turning around. Phasic and mean blood pressure was measured and heart rate was derived electronically from phasic blood pressure. Both variables were recorded continuously on a Beckman R612 Dynograph.

Dose-response curves for the effect of acetylcholine (ACh), noradrenaline (NA), phenylephrine (Phen) and angiotensin II (AII) were obtained in both cholesterol-fed and normal animals; 3–5 doses of each agent were selected, so as to achieve a change in blood pressure of at least 20% in the less responsive group. The same animals were used for all experiments with an interval of at least two days between experiments.

The influence of cholesterol feeding on the drug effects was assessed by comparing the drug-induced blood pressure changes in the two groups (atherosclerotic vs normal rabbits) by analysis of covariance using the logarithmic dose as a covariate (Multiple General Linear Hypothesis Module of SYSTAT). Calculations were performed with the SYSTAT programme and P values < 0.05 for differences between groups were considered significant.

When all *in vivo* experiments had been performed, the rabbits were anaesthetized with pentobarbitone and killed by exsanguination. The descending thoracic aorta was excised immediately, cleared of connective tissue and cut into rings approximately 2–3 mm wide. Great care was taken to avoid damage to the endothelium and the presence of an ACh-induced relaxation in each ring of the control group was accepted as evidence that our tissue handling did not damage the endothelium. These rings were suspended in 10 ml organ baths containing a Krebs-Henseleit (KH) solution of the following composition (mM): NaCl 118.0, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and glucose 10.1. The solution was kept at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2. The pH was determined with an IL 613 blood gas analyzer (Instrumentation Laboratory Inc. Lexington, Massachusetts 02173) and adjusted to 7.4 by adding small amounts of either $NaHCO_3$ or HCl. The tension of the rings was recorded isometrically with electromechanical transducers (Statham UC 3) on a potentiometric recorder. At the beginning of the experiments the rings were stretched to an initial tension of 1 g and allowed to adjust for 1 h. The bathing medium was changed every 15 min to prevent accumulation of metabolites.

ACh-induced relaxation was assessed in rings precontracted with 1 μM NA in the presence of 1 μM propranolol. When the maximal effect of ACh had been reached, a dose of 10 μM sodium nitroprusside was added to assess the maximal degree of relaxation achievable for each ring. The effects were expressed as percentages of this maximum response (= 100%). Effects in normal and atherosclerotic rings were compared by the non-parametric test of Mann-Whitney and differences were accepted for P values < 0.05.

Drugs

The following were used: (−)-noradrenaline bitartrate, (Hoechst); acetylcholine chloride (Sigma), propranolol HCl (ICI); isradipine (Sandoz), (−)-phenylephrine HCl (Sigma); angiotensin II (Hypertensin, Ciba-Geigy) and sodium nitroprusside (Fluka).

For the experiments *in vivo*, isradipine was dissolved in 94% ethanol and polyethylene glycol 400, 0.01 ml and diluted with 5% glucose to 1 ml per 100 μg active drug. This dose, i.e. 100 $\mu g\,kg^{-1}$, was chosen after consideration of previous experiments in conscious rabbits (Hof, 1987a) and was infused during 10 min. A further 5 min were allowed for the haemodynamic variables to stabilize, then the dose-response curves to ACh or the pressor agents were re-evaluated. All other drugs were dissolved in 0.9% NaCl and diluted to the required volume with 5% glucose. The injection of the vehicles at the volumes used (up to a total of 1 $ml\,kg^{-1}$) had no effect on heart rate nor blood pressure. For the experiments *in vitro*, substances were dissolved in isotonic saline.

Results

Rabbit aorta in vitro

The aortae of all animals receiving the cholesterol diet were macroscopically severely atherosclerotic. Figure 1 shows that the relaxant response to ACh was almost totally lacking in aortic rings of cholesterol-fed animals precontracted with noradrenaline. Nitroprusside relaxed normal and atherosclerotic vessels and its effect was used as 100% value in order to normalize the relaxant effect of ACh. These experiments indicate severe endothelial dysfunction in the cholesterol-fed animals used for the experiments described in the next section.

Conscious rabbits

Figure 2 shows the dose-response curve to ACh in conscious rabbits. No significant differences between cholesterol-fed and control rabbits were observed.

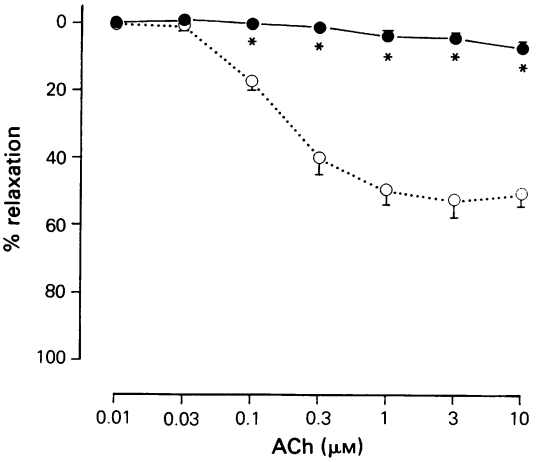

Figure 1 Relaxant effects of acetylcholine (ACh) in noradrenaline (NA) precontracted aortic rings from atherosclerotic (●) and normal (○) rabbits expressed as a percentage of the response obtained with 10 μM sodium nitroprusside. Mean of 18 rings for each point, bars show s.e.mean, asterisks show significant ($P < 0.05$) differences between atherosclerotic and normal rabbits.

Isradipine (100 $\mu g\,kg^{-1}$) decreased blood pressure by 23 mmHg (29.6%) in normal and 30 mmHg (31%) in atherosclerotic animals (the blood pressure of atherosclerotic animals was higher: 96 ± 4.3 vs. 77 ± 2.1 mmHg in the control animals). It shifted the dose-response curve to ACh to the right in both atherosclerotic and normal rabbits and again there was no significant difference attributable to cholesterol feeding.

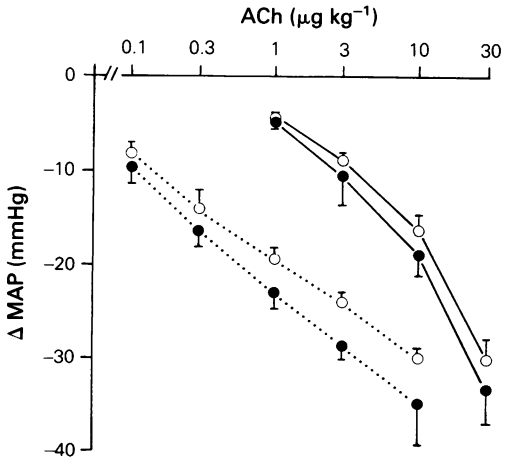

Figure 2 Decrease in mean arterial pressure (MAP, mmHg) induced by the injection of acetylcholine (ACh) into conscious atherosclerotic (●) and normal (○) rabbits before (dotted lines) and after (continuous line) injection of isradipine 100 $\mu g\,kg^{-1}$. $n = 6$ for each group, bars show s.e.mean.

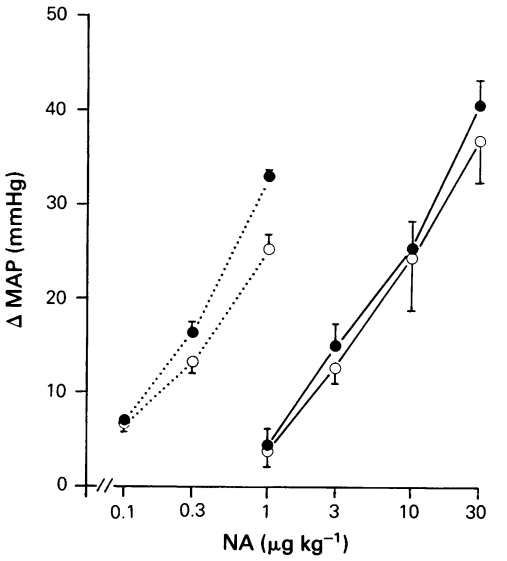

Figure 3 Increases in mean arterial pressure (MAP, mmHg) induced by the injection of noradrenaline (NA) into atherosclerotic (●) and normal (○) conscious rabbits before (dotted lines) and after (continuous line) injection of isradipine 100 $\mu g\,kg^{-1}$. $n = 6$ for each group, bars show s.e.mean.

Figure 3 shows the dose-response curves to NA. The analysis of covariance indicates that the curve was significantly steeper in cholesterol-fed rabbits. Isradipine shifted the dose-response curve by a factor of about 10 to the right and abolished the difference between atherosclerotic and control rabbits.

Figure 4 shows the same type of experiment using the selective α_1-adrenoceptor stimulating agent Phen. Again cholesterol-fed animals showed a significantly enhanced pressor response. Isradipine pretreatment shifted the dose-response curve to the same extent to the right as in the experiments with NA and it abolished the difference between cholesterol-fed and normal rabbits.

The cholesterol-fed rabbits showed an enhanced pressor response to AII (Figure 5). After isradipine pretreatment the dose-response curves were shifted to the right as with the other agents, but the increased responsiveness indicated by a significantly steeper slope of the dose-response curve of cholesterol-fed rabbits persisted.

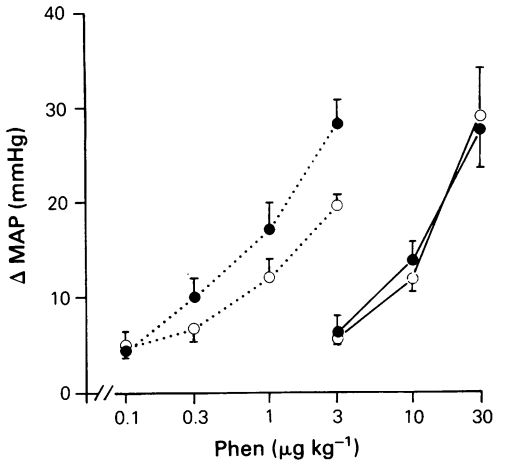

Figure 4 Increase in mean arterial pressure (MAP, mmHg) induced by the injection of phenylephrine (Phen) into atherosclerotic (●) and normal (○) conscious rabbits before (dotted lines) and after (continuous line) injection of isradipine 100 $\mu g\,kg^{-1}$. $n = 6$ for each group, bars show s.e.mean.

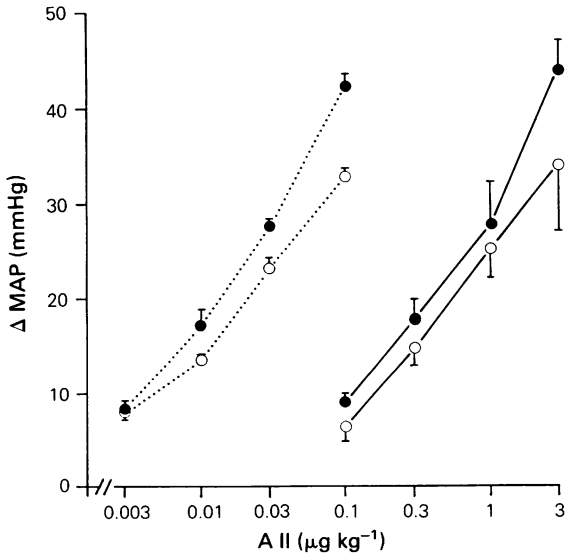

Figure 5 Increase in mean arterial pressure (MAP, mmHg) induced by the injection of angiotensin II (AII) into conscious atherosclerotic (●) and normal (○) rabbits before (dotted lines) and after (continuous line) injection of isradipine 100 $\mu g\,kg^{-1}$. $n = 6$ for each group, bars show s.e.mean.

Discussion

Changes of BP reflect the integrated response of the whole circulation, with resistance vessels probably contributing most. With intra-arterial injection of ACh regional vasoconstriction was observed in cholesterol-fed rabbits (Bossaller *et al.*, 1987b) and in man (Ludmer *et al.*, 1986). With intravenous injection Wright & Angus (1986) have observed that ACh-mediated dilatation persisted in attenuated form in the lower body of atherosclerotic rabbits. Our results indicate that endothelium in resistance vessels of atherosclerotic animals responds normally to ACh, despite the aortae of the same animals showing a severe lack of endothelial relaxation *in vitro*. Our results demonstrate that ACh-induced hypotension is not useful for detecting atherosclerosis *in vivo*.

Like other investigators we have found that atherosclerosis alters pressor responses to constrictor agents (Rosendorff *et al.*, 1981; Heistad *et al.*, 1984). We have used the nonselective α-adrenoceptor stimulant NA, the α_1-adrenoceptor selective stimulant Phen and angiotensin II. The pressor response to all three agents was similarly enhanced in atherosclerotic animals suggesting a mechanism which does not discriminate between various agonists. Attempts have been made to clarify this hyperresponsiveness of arteriosclerotic vessels by experiments *in vitro*, but the results differed greatly depending on species and experimental conditions (Henry & Yokoyama, 1980; Godfraind & Miller, 1983; Kawachi *et al.*, 1984; Ginsburg *et al.*, 1984; Harrison *et al.*, 1987). The defective endothelium-derived relaxing factor (EDRF) release in atherosclerotic vessels (Freiman *et al.*, 1986; Jayakody *et al.*, 1987a; Bossaller *et al.*, 1987a) could contribute to the enhanced pressor responses, even though we have not found a diminished response to ACh (it appears unlikely that ACh could play an important physiological role *in vivo*). Alternatively, it is conceivable that atherosclerosis or hypercholesterolemia could enhance the production of endothelium-derived contracting factor (EDCF) or endothelin (Yanagisawa *et al.*, 1988) as is found in hypertension or in regenerating endothelium (Luescher & Vanhoutte, 1986; Vanhoutte, 1987; Shimokawa *et al.*, 1987).

Calcium antagonists are well known to antagonize vasoconstrictor effects and there is evidence for differences between various constrictor agents (Hof, 1987b). The response of atherosclerotic animals has not been investigated so far and we used the potent and selective dihydropyridine derivative, isradipine (Hof *et al.*, 1987), for this purpose. The calcium antagonist shifted the dose-response curves to NA and Phen (but not AII) more effectively to the right in atherosclerotic animals so that the pressor

response for the former two agents became similar in both groups of animals.

In summary, our experiments support the view that pressor effects of several vasoconstrictor agents are enhanced in atherosclerotic animals. The difference between normal and atherosclerotic animals is, however, small and certainly not sufficient to form the basis for a diagnostic test. The response to acetylcholine was not blunted in atherosclerotic animals even though the response of aortic rings of the same animals *in vitro* was almost totally lacking. It is likely that small resistance vessels, which contribute most to changes in blood pressure *in vivo*, are affected by atherosclerosis (and/or hyperlipidemia) but differ from large arteries in their responsiveness. Indeed, atherosclerosis induced alterations of vascular responsiveness are known to change even along the aorta (Verbeuren *et al.*, 1986). It would be interesting to investigate resistance vessels from atherosclerotic animals *in vitro* in order to investigate the mechanisms underlying these changes in responsiveness.

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