The lipids present in these 2 lipoprotein fractions were found to be virtually identical (figure 2). Diglyceride was the major non-polar lipid class (60-70%). Other non-polar lipids present were triglycerides (8-10%), hydrocarbons (10-15%), and free fatty acids (5%). Small amounts of sterol esters and free sterols were also present (figure 2, A and B). The major phospholipids present included phosphatidyl cholines and phosphatidyl ethanolamines (figure 2, C and D), along with sphingomyelins, and unidentified polar (figure 2, 'P') and apolar lipids, including biochromes (figure 2, 'A'). The lipid profile of the 2 major lipoprotein fractions was similar to that of whole plasma, suggesting that haemolymph lipids are transported as lipoproteins. Since more 1,3-diglyceride than 1,2-diglyceride was found in the lipoproteins than in whole plasma, some 1,2-diglyceride may be converted to 1,3-diglyceride during electrophoresis.

1 This study is a contribution of the Missouri Agricultural Experiment Station, Journal series No. 8520.
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Differential sensitivity of the two phases of ear artery contraction to intimal and adventitial norepinephrine[1]

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Summary. The biphasic contraction of the rabbit ear artery to norepinephrine (NE) was investigated in the normal (adventitial stimulation) and the everted (intimal stimulation) segment of ear artery. The 2nd phase response showed an intimal ED_{50} of 8.2×10^{-8} M which was significantly ($p < 0.05$) lower than the adventitial ED_{50} of 42.6×10^{-8} M. This difference was abolished by inhibition of neuronal and extraneuronal uptake for NE. The 1st phase response also showed an ED_{50} for the intimal stimulation (6.9×10^{-8} M) which was significantly ($p < 0.05$) lower than adventitial (65.5×10^{-8} M). This difference was reduced but not abolished by NE uptake inhibition. This suggsets that some feature of the adrenergic neuroeffector apparatus is asymmetrically arranged to favor fast responses to blood borne NE.

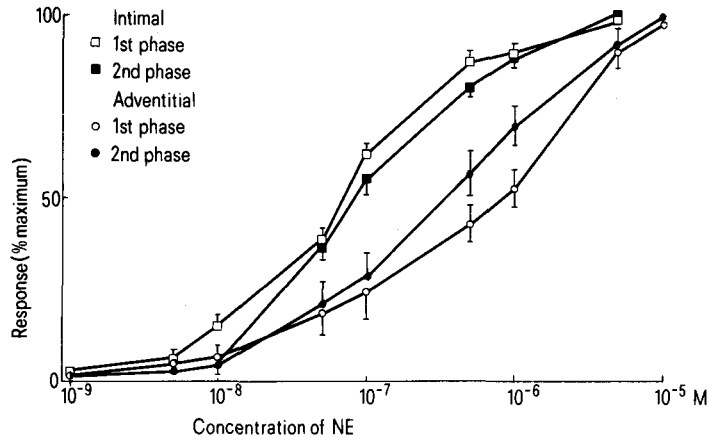
The response of vascular smooth muscle to norepinephrine (NE) is biphasic[2-4]. The 1st phase is due to release of calcium from a bound or intracellular store while the final contraction is due to influx of calcium from the external medium[5]. Norepinephrine when applied to the intimal surface of the arteries elicits the steady-state final contraction with a greater efficacy than when applied to the adventitial surface and this difference is abolished by blockade of neuronal and extraneuronal uptake mechanisms[6,7]. Recent work suggests that the 1st phase of the NE contraction shows a similar asymmetric difference which is not due to uptake mechanisms[7-9]. The present study investigated this suggestion over the complete concentration-response curve for NE.

A 15-mm segment of the central ear artery was removed from New Zealand white rabbits which had been stunned and killed by exsanguination. This was transferred to a dissection bath containing Krebs bicarbonate solution gassed with 95% O_2 and 5% CO_2. Two 4-mm segments were prepared for in vitro tension recording as previously described[6]. One of the segments was prepared in the normal configuration with the adventitia outward. The lumen was filled with petroleum jelly and this segment was used to investigate the effects of drugs applied only to the adventitial surface. The 2nd segment was everted in the manner previously described[6]. The lumen of the everted segment was filled with the waterproof petroleum jelly and this preparation was used to investigate the effects of drugs applied only to the intimal surface of the vessel.

The responses to the adventitial and intimal stimulation with NE were investigated. NE was added to the tissue bath to give final concentrations of between 10^{-9} M and 10^{-5} M.

The mean ED_{50} concentration (M) for the 1st and 2nd phases of NE contractions are shown in control and in uptake blockaded arteries. The range in brackets defines the 95% confidence interval. The asterisk denotes a significant difference between intimal and adventitial values

	Control	DMI+DOC
Intimal NE		
1st phase	6.9×10^{-8} (6.3–7.7)	7.1×10^{-8} (5.8–9.2)
2nd phase	8.2×10^{-8} (7.2–9.4)	6.5×10^{-8} (5.1–8.3)
Adventitial NE		
1st phase	65.5×10^{-8}* (47.8–125.1)	13.0×10^{-8}* (9.5–20.9)
2nd phase	42.6×10^{-8}* (29.6–76.1)	4.4×10^{-8} (3.8–5.1)



Intimal and adventitial dose-response curves. The NE concentration-response curves for both the 1st [open symbols] and the 2nd [closed symbols] phases of the ear artery contraction are shown. The response-curves when NE is applied to the intimal surface [squares] are to the left of those for the adventitial surface [circles]. The greatest difference is shown for the 1st phase of the contraction.

The response to each concentration was allowed to reach a steady-state contraction and was then washed from the bath. After a 15-min interval a 2nd NE concentration was tested. In this way the amplitude of the initial and final phases of contraction were determined for each NE stimulus.

The final contraction amplitude was readily obtained from each response, and this was taken as an index of the 2nd phase of contraction[3-6]. The amplitude of the initial phase was measured as the amplitude of contractile force at the 1st point after the beginning of the contraction where there was a pause in the initial linear increase in tone. This point was often associated with a slight relaxation in tension.

The maximum amplitude of each of these phases together with the NE concentration which produced half maximal response (ED_{50}) were identified in each experiment. 2 kinds of experiment were carried out. Adventitial and intimal responses to NE were compared in normal ear arteries, and in arteries where neuronal and extraneuronal uptake of NE were blocked with desmethylimipramine (DMI, 10^{-7} M) and deoxycorticosterone (DOC, 3×10^{-5} M).

The control 1st and 2nd phase responses are shown in the figure. The initimal 2nd phase steady-state contraction [closed squares] displayed a response curve which was significantly displaced to the left of the adventitial second phase contraction [closed circles]. This observation is similar to that already reported in other studies[6,7,10]. The relationship between the response curves for the initial response shows an even greater separation. The adventitial 1st phase response [open circles] was displaced significantly to the right of the adventitial steady-state response [closed circles]. In contrast, the intimal 1st phase response [open squares] was slightly displaced to the left of the intimal steady-state response curve [closed squares]. This suggests that the intimal surface preferentially elicits the rapid 1st phase response whereas the adventitial surface elicits the slow final steady-state response. The computed mean ED_{50} values are shown in the control column of the table and support this view.

Application of DMI and DOC to the tissues results in blockade of neuronal and extraneuronal uptake mechanisms for NE. This had the effect of reducing the differences between the intimal and adventitial routes of NE application. The steady-state 2nd phase of the NE response in the adventitial vessel was shifted to the left of its position in the figure until it was not significantly different to the results with intimal application of the NE (table). However, the 1st phase of contraction to the adventitial NE remained significantly offset to the right of those intimal curves and displayed a higher ED_{50} of 13.0×10^{-8} M (table). These results show that the NE uptake mechanisms are particularly important in limiting the movement of NE from the adventitial surface into the media. Thus the present work with vessel segments correlates well with studies of the NE responses of perfused ear arteries. These perfusion studies showed that the steady-state contraction is potentiated best by blockade of neuronal uptake when the NE is applied extraluminally to the adventitia[10]. Further addition of an extraneuronal uptake inhibitor potentiated the extraluminal NE to a greater extent than the intraluminal NE[8].

Therefore, NE uptake mechanisms act as a barrier to the diffusion of NE through the tissue and limit the slower 2nd phase response. The 1st phase response is also limited by this uptake barrier as the adventitial ED_{50} for the 1st phase was reduced by uptake inhibition. However, some further limiting factor remains to prevent the adventitial 1st phase ED_{50} reaching that found at the intima. Previous work using the aorta[9] shows that this factor is unlikely to be the structural barrier posed by the adventitial tissue as similar intimal/adventitial response differences were found when the adventitia was stripped from the vessel. It is possible that the residual asymmetry in the fast phase response is due to some other adventitial/medial NE binding or metabolic barrier which is not associated with the uptake mechanisms. In any case it seems likely that the adrenergic receptor mechanisms responsible for the first phase of contraction are preferrentially located towards the intimal surface ensuring a rapid response to blood-borne NE.

1 Supported by American Heart Association-Greater Los Angeles Affiliate Grant No. 602. We wish to thank Dr John Bevan and Dr Alasdair MacLean for helpful advice.

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