Br. J. Pharmac. (1975), **54**, 421-427

THE EFFECTS OF HYPERCHOLESTEROLAEMIC PLASMA ON VASCULAR SENSITIVITY TO NORADRENALINE

D. BLOOM, T.A. McCALDEN & C. ROSENDORFF

Department of Physiology, University of the Witwatersrand Medical School, Hospital Street, Johannesburg, South Africa

1 The pressor responses to injected noradrenaline (NA) of 42 isolated perfused femoral arteries of the rabbit were studied.

2 Potentiation of the responses was found when hypercholesterolaemic plasma was perfused through the arteries. No change was found with normal plasma.

3 Potentiation of the responses was found when isolated β-lipoprotein in Krebs solution was perfused. No change was found with similar amounts of bovine albumen.

4 Pure cholesterol dissolved directly into normal plasma, and dissolved via propanol into Krebs solution or plasma caused no potentiation. Propanol alone in Krebs or plasma had no effect.

5 Potentiation was caused by a decreased equilibrium coefficient (K_{eq}) for the NA-adrenoceptor interaction and an increased maximal pressor response (R_{max}).

6 It is concluded that cholesterol carried on its apoprotein is capable of potentiating the pressor effects of noradrenaline.

Introduction

It has been demonstrated that many steroids are capable of inhibiting the extraneuronal uptake of noradrenaline (NA) and related compounds in heart muscle (Iversen & Salt, 1970). Subsequently Salt & Iversen (1972) have demonstrated that cholesterol (when dissolved in alcohol and added to saline) is capable of a similar type of inhibition. Although the sensitivity of the heart muscle to NA was not studied, inhibition of the uptake processes would be expected to potentiate the effects of the hormone.

If the effects of cholesterol on vascular smooth muscle are similar to those in heart muscle an elevation of blood cholesterol levels should produce an increased vascular response to sympathetic activity. Burstyn, Horrobin & Miurin (1972) have shown that intravenous infusion of a sonicated cholesterol solution causes an increase in arterial blood pressure. Also, we have previously demonstrated that in obstructive jaundice where the serum cholesterol levels are elevated (Sherlock, 1968), there is a potentiated pressor response to injected noradrenaline (Bloom, Bomzon, Rosendorff & Scriven, 1974; Bloom, McCalden & Rosendorff, 1974). This circumstantial evidence would suggest that cholesterol is capable of causing a potentiated vascular response to noradrenaline.

Cholesterol is mainly carried in plasma on the β-lipoprotein and elevated levels of this protein are found in Fredrickson's type IIA hyperlipidaemia (Fredrickson, Levy & Lees, 1967).

We have, therefore, investigated the effects on the pressor response to NA of cholesterol in Krebs solution, cholesterol in hyperlipidaemic plasma and a β-lipoprotein extract. We have also attempted to dissolve cholesterol directly in normal plasma since sonication or dissolution in ethanol is unphysiological.

Methods

The arterial model used to assay the vasoconstrictor effect of NA was similar to that described by de la Lande & Rand (1965). The technique involved the measurement of perfusion pressure in a constant flow system.

New Zealand White rabbits were anaesthetized with intravenous pentobarbitone sodium (Nembutal, Abbott). A length (2-2.5 cm) of femoral artery was exposed, cannulated proximally, removed from the animal, washed through with a Krebs solution containing heparin and then perfused at constant flow in an organ bath with Krebs solution at 37° C. The bath was maintained

at this temperature with an outer thermostatically controlled water jacket. The Krebs solution contained (mM) NaCl 118.0, KCl 4.69, NaH_2PO_4 1.33, $NaHCO_3$ 25.0, glucose 5.56, $CaCl_2$ 2.52 and $MgCl_2$ 1.05 and all perfused solutions were maintained at pH 7.40 and constant PCO_2 and PO_2 by bubbling a gas containing 6% CO_2 and 94% O_2 through the perfusate reservoir. Perfusion pressure was monitored with a Statham P 23 BB transducer connected to the flow system upstream of the artery via a T tube, and the perfusion rate was kept constant throughout each experiment at 4-5 ml/minute. This gave a perfusion pressure of 5-10 mmHg in the system.

The arterial constrictor responses to NA were determined by injection of graded doses of NA in Krebs solution at 37°C just proximal to the preparation. The resultant changes in vessel calibre and resistance were monitored as changes in perfusion pressure and recorded on a Beckman dynograph.

The system was allowed to stabilize for at least 30 min during perfusion with Krebs solution. Then a series of responses to graded doses of NA was obtained. The perfusate was then changed to the test solution, allowed to stabilize for 30 min and a further series of responses to NA was obtained. The perfusate was again changed to Krebs solution and 30 min later another series of responses was obtained.

Hyperlipidaemic plasma

Blood (60 ml) was taken from each of 15 fasting patients attending a special clinic for hyperlipidaemia. The only therapy was a diet and cholestryamine. All patients had elevated serum cholesterol levels and in each case it was confirmed by both ultracentrifugation and electrophoresis that these patients were true type IIA hyperlipidaemics (Fredrickson *et al.*, 1967). The blood was centrifuged for 3 min at 2500 rev/min and the supernatant plasma removed. Plasma urea and electrolytes were measured in each sample. In each experiment three of the plasma samples were selected at random, pooled and analysed for total cholesterol, β-cholesterol and triglycerides. Six experiments with hypercholesterolaemic plasma (HC) were performed and five with plasma from normal patients as controls.

Cholesterol in Krebs solution

Cholesterol (B.D.H.) was dissolved in propanol to give a stock solution of 50 mg/ml. The aliquots of this stock were added to Krebs solution to make a final concentration of 10 μg/ml to 50 μg/ml. Higher concentrations were not used because the cholesterol precipitated out of solution; five experiments were carried out. Three controls with propanol alone were also carried out.

β-Lipoproteins

Normal human plasma was spun in a Beckman C-265B ultracentrifuge at 19,000 rev/min for a period of 30 min to remove the chylomicrons. Then the samples were spun at 40,000 rev/min for 18 h with a density solution containing 11.4 g NaCl, 0.1 g disodium edetate (EDTA) and 1 ml 0.1 N NaOH made up to 1 litre with distilled water and of specific gravity 1.006. Pre-β-lipoproteins moved from plasma into this solution and were removed. The remaining plasma was further spun at 40,000 rev/min for 20 h with a second density solution containing 24.98 g NaBr in 100 ml of the first density solution and of specific gravity 1.182. The β-lipoproteins moved from plasma into this solution and their presence was confirmed with a Technicon AII autoanalyser as well as by electrophoresis. The β-lipoproteins were then dialysed against 0.9% w/v NaCl solution (saline) for 24 h to remove the EDTA and bromide. By this procedure the β-lipoprotein content in 600 ml of plasma was concentrated into 200 ml. This concentrate was then added to an equal volume of Krebs solution to give final concentrations of β-cholesterol 0.75 to 1.1 mg/ml. Five experiments were carried out in which these solutions were infused into femoral artery preparations, and an equal number of controls with bovine albumen (Merck) at 0.5 to 2.0 mg/ml.

Cholesterol in plasma

Cholesterol was added to normal human plasma either directly or from the propanol stock solution previously described. A maximum of 10 μg/ml of the cholesterol could be dissolved directly and 30 μg/ml with the propanol stock. Ten such experiments were carried out. Three controls with propanol alone in plasma were also performed.

Analysis of results

Two methods of analysing these results were used.

Method 1 The dose-response data obtained in each experiment were plotted as the increase in perfusion pressure (Response mmHg) against the logarithm of the dose (μg NA). This produced three curves for each experiment, one for control (Krebs) perfusate, one for the experimental perfusate and one for the washout with Krebs solution. The responses obtained when the plasma

was perfused was compared to those of the first Krebs perfusion by a paired t test.

Method 2 The dose-response data obtained in each individual experiment were plotted as the reciprocal of the perfusion pressure increase (1/Response, mmHg) against the reciprocal of the dose (1/dose of NA, μg). This produced a straight line relation in each experiment as predicted from Michaelis-Menten kinetics for drug-receptor interaction (Goldstein, Aronow & Kaplan, 1965).

$$\frac{1}{R} = \frac{K_{eq}}{R_{max}} \cdot \frac{1}{D} + \frac{1}{R_{max}}$$

where R = response (mmHg), D = NA dose (μg), R_{max} = maximal response (mmHg), K_{eq} = equilibrium coefficient for drug-receptor interaction.

In each experiment values of R_{max} and K_{eq} were calculated from the intercepts of the line on the x and y axes respectively. When $1/D$ is zero, $1/R$ (y intercept) is equal to $1/R_{max}$. When $1/R$ is zero, $1/D$ (x intercept) is equal to $-1/K_{eq}$. Changes in $1/R_{max}$ imply changes in the intrinsic activity of the drug-receptor complex, while changes in $-1/K_{eq}$ imply changes in drug-receptor affinity.

Results

Hyperlipidaemic plasma (HC plasma)

Figure 1 shows three sets of responses obtained from a femoral artery. The top trace shows the pressure changes in the perfusate after injection of doses of NA ranging from 0.8 μg to 8.0 μg. The middle trace shows the pressure changes after injections of doses of NA ranging from 1.6 μg to 8.0 μg when the artery was perfused with HC plasma and the bottom trace after a period of washout with Krebs solution. It is clear that the responses to NA are much larger in the presence of the HC plasma. Table 1 shows the individual responses obtained in each of the six experiments before and during perfusion with HC plasma. Using a paired t test the responses with HC plasma were found to be significantly different from the first responses in Krebs solution ($P < 0.01$), in all of the six experiments. When normal plasma was perfused no potentiation was seen.

Another similar experiment is shown in Figure 2 as a log-dose *vs* response plot. The potentiation of the NA response in the presence of HC plasma is seen as a shift of the curve to the left of the control curve. After washout with Krebs solution the dose-responses were shifted back towards (but never quite reached) the initial curve.

Figure 3 shows a double reciprocal plot of the data from a typical experiment. In all of the

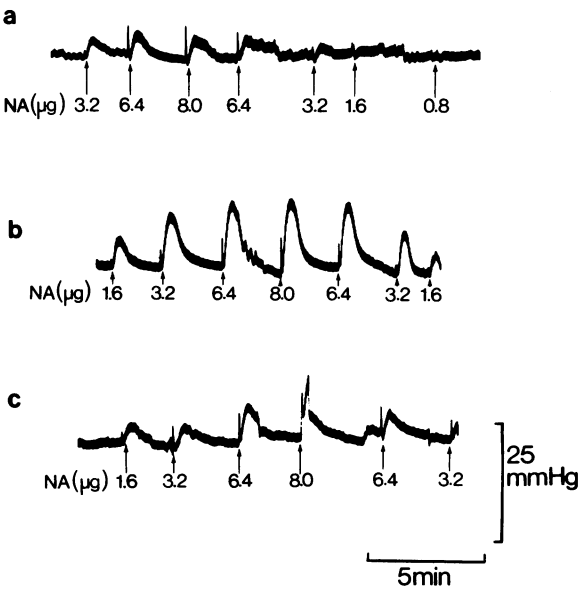

Figure 1 Three traces of perfusion pressure in isolated femoral arteries of the rabbit are shown. The top trace (a) shows the responses as changes in perfusion pressure (mmHg) when several doses of noradrenaline (NA) were injected during perfusion with Krebs solution. Trace (b) shows similar responses when hypercholesterolaemic plasma (HC plasma) was perfused and trace (c) those obtained during reperfusion with Krebs solution. A time scale (min) and a pressure calibation are also shown. HC plasma potentiates the effects of NA.

experiments a high correlation coefficient was obtained (0.78–0.98). It can be seen that both the slope and the x intercept obtained with Krebs solution are different from those obtained during perfusion with HC plasma, whereas the y intercept is not significantly changed. Thus, the maximum response (R_{max}) of this artery has remained almost unchanged while there was a large change in the drug-receptor equilibrium coefficient (K_{eq}) (Goldstein *et al.*, 1968). Table 2 shows the values of K_{eq} and R_{max} for all HC experiments. Using a paired t test the K_{eq} (HC) was found to be significantly different ($P < 0.05$) from the K_{eq} (Krebs). Although there is a tendency for the R_{max} to be increased with the HC plasma this was not significant at the 5% level. There was no significant change in K_{eq} or R_{max} in the normal plasma controls. The assayed values of total cholesterol and % β-cholesterol are also shown in Table 2. There is no correlation between the degrees of NA potentiation and the total cholesterol concentrations.

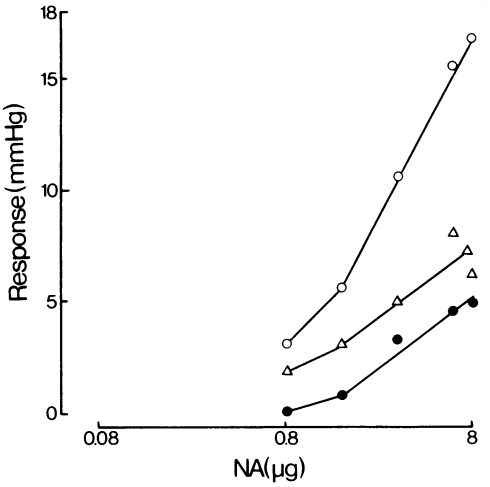

Figure 2 The pressure responses (mmHg) on the *y* axis are plotted against the log of the dose of noradrenaline (NA, μg) on the *x* axis. The responses obtained during hypercholesterolaemic plasma perfusion (○) are shifted to the left of those during initial Krebs solution perfusion (●). Washing with Krebs solution (△) caused partial reversal of this effect.

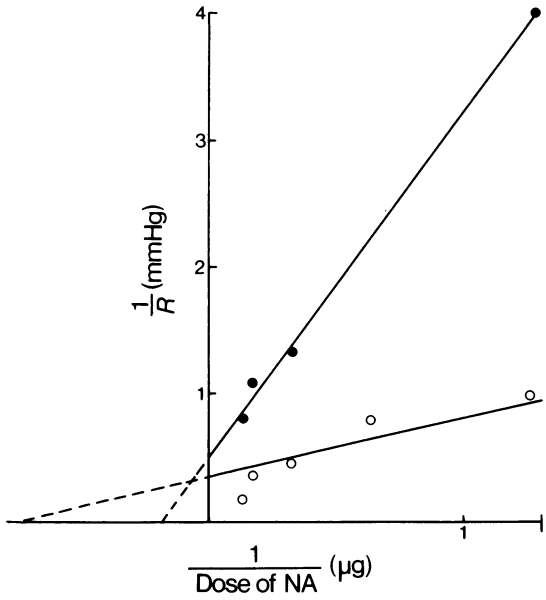

Figure 3 The reciprocal of response (mmHg) on the *y* axis is plotted against the reciprocal of the dose of noradrenaline (NA, μg) on the *x* axis. The slope and the *x* intercept obtained during hypercholesterolaemic plasma perfusion (○) are different from those obtained during Krebs solution perfusion (●). There is no difference in the *y* intercepts. Thus the K_{eq} value has changed but the tissues maximal response (R_{max}) has not.

Cholesterol in Krebs solution

In the five experiments carried out no potentiation of the response to noradrenaline was found at any of the concentrations (10 μg/ml to 50 μg/ml) used.

Table 1 The perfusate pressure changes (mmHg) in six experiments when noradrenaline was injected in doses ranging from 0.8 to 8.0 μg. (The values are shown during Krebs (K) and during hypercholesterolaemic plasma (HC) perfusion)

		NA dose (μg)					
Expt. no.		*0.8*	*1.6*	*3.2*	*6.4*	*8.0*	
1	K	0	1.2	3.4	4.7	5.0	$P < 0.01$
	HC	2.5	5.3	10.6	14.7	17.5	
2	K	0	0.5	0.7	1.3	1.5	$P < 0.01$
	HC	1.0	1.7	3.8	5.0	5.0	
3	K	1.8	2.9	3.7	6.6	8.8	$P < 0.01$
	HC	5.1	10.3	16.9	19.8	22.1	
4	K	0.2	1.0	0.7	0.9	1.2	$P < 0.01$
	HC	1.1	1.3	2.1	2.7	6.2	
5	K	6.3	7.5	16.9	30.0	–	$P < 0.01$
	HC	15.0	33.7	43.7	–	–	
6	K	0.9	2.5	4.7	6.9	6.6	$P < 0.025$
	HC	8.4	13.8	18.8	–	–	

The results of paired *t* tests are shown on the right of the table.

β-Lipoproteins

Figure 4 shows the responses obtained to NA in an isolated artery before, during and after perfusion with 1 mg/ml of β-lipoproteins. Although the doses of NA used with lipoprotein were one tenth of those in Krebs solution, much larger responses were obtained. This would indicate a shift of the log dose-response curve to the left during protein perfusion. Comparison by a paired t test of the responses in Krebs solution to the responses when β-lipoprotein was added, showed a significant difference in all five experiments ($P < 0.01$).

Table 3 shows the calculated values of K_{eq} and R_{max} (from a Lineweaver-Burke plot) for all five experiments with β-lipoprotein. A decrease in K_{eq} and an increase in R_{max} occurred. No change in sensitivity to NA occurred in any of the controls when bovine albumen was used.

Cholesterol in plasma

The maximum possible concentration of cholesterol produced no potentiation of the response to NA in any of the 10 experiments carried out. We found that propanol added either to Krebs solution or to normal plasma had no effect on the vascular response to NA.

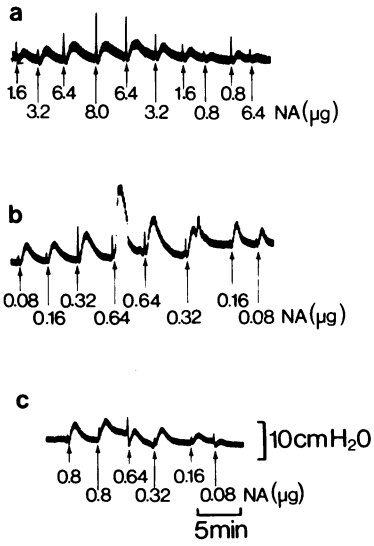

Figure 4 The responses to noradrenaline (NA) obtained in one experiment are shown (a) during Krebs solution perfusion; (b) during β-lipoprotein perfusion (1 mg/ml), and (c) after a washout of the β-lipoprotein with Krebs solution. Time and pressure calibrations are shown. It can be seen that the lipoprotein potentiates the response to NA.

Table 2 The equilibrium coefficient (K_{eq}) and the maximal possible response (R_{max}) calculated for each hypercholesterolaemic plasma experiment and each normal plasma control

			Hypercholesterolaemia experiments			
	Total cholesterol		K_{eq}		R_{max}	
Expt. no.	*mg/100 ml*	*β-cholesterol %*	*Krebs*	*HC Plasma*	*Krebs*	*HC Plasma*
1	525	75	37.1	27.7	30.3	90.9
2	415	72	6.6	8.7	2.5	11.9
3	370	70	3.4	5.5	9.4	41.7
4	326	71	5.3	2.4	1.9	4.1
5	490	70	4.0	0.1	34.5	4.6
6	300	73	7.4	2.3	14.3	32.3
				$P < 0.05$		NS
			Normal plasma controls			
1	170	65	15.0	2.9	12.5	1.5
2	165	69	8.9	2.9	33.3	16.6
3	200	70	1.1	2.2	4.3	5.3
4	195	65	2.7	8.0	25.0	27.7
5	201	68	2.9	4.2	6.7	9.1
				NS		NS

The significance (paired t test) of any changes are shown at the base of each column. Also shown are the values of total cholesterol (mg%) and the % β-cholesterol for each of the plasmas used.

Table 3 The values of the equilibrium coefficient (K_{eq}) for the noradrenaline (NA)-receptor interaction and the maximal response (R_{max}) to NA in the five experiments when Krebs solution was followed by perfusion with a β-lipoprotein solution.

	K_{eq}		R_{max} (cmH_2O)	
Expt. No.	*Krebs*	*β-protein*	*Krebs*	*β-protein*
1	0.85	0.19	5.08	16.95
2	5.14	5.86	22.73	71.43
3	2.49	0.75	11.49	15.53
4	2.42	1.44	13.89	55.56
5	13.89	2.23	52.63	58.82
Mean	4.96	2.09	21.16	43.66

Discussion

When pure cholesterol was either dissolved in plasma or in Krebs solution no potentiation of the effects of noradrenaline (NA) were observed. The concentrations used were in excess of those used by Salt & Iversen (1972) in heart muscle but lower than those found pathologically. Salt & Iversen reported that the extraneuronal uptake of NA into heart muscle was inhibited. Our failure to demonstrate potentiation of the responses to NA in the vascular preparation may mean that the vascular muscle is not as sensitive to cholesterol as heart muscle and that much larger concentrations of cholesterol are needed.

Large increments of plasma cholesterol are found in Fredrickson's type IIA hyperlipidaemia (Fredrickson *et al.*, 1967). This is largely carried in plasma as an increase in the β-lipoprotein fraction. When plasma from these patients was used, potentiation of the responses to NA was obtained. This potentiation might have been achieved either because the concentration of cholesterol was much higher than in the previous experiments; or because when cholesterol is attached to its apoprotein it is much more effective. There may also have been some other factor in the hyperlipidaemic plasma which potentiated the response to NA. However, this seems unlikely as our experiments with isolated β-cholesterol (attached to β-lipoprotein) would indicate that this constituent of the plasma was primarily responsible for the potentiation. This would strengthen the hypothesis that cholesterol attached to its apoprotein is more effective than pure dissolved cholesterol.

The analysis of the data by Michaelis-Menten kinetics (Goldstein *et al.*, 1968) indicates that when the vascular muscle is exposed to a high cholesterol concentration there is a decreased equilibrium coefficient (K_{eq}) for the NA-adrenoceptor interaction and an increased maximal response (R_{max}). The decrease in K_{eq} would imply that more NA-adrenoceptor complex was formed for a given dose of NA. Thus, either the affinity of the receptors for NA was increased or more of the injected NA became available for combination at the receptor site. An inhibition of extraneuronal uptake of NA into the vascular muscle would result in more NA becoming available for receptor combination. In view of the work of Salt & Iversen (1972) this mechanism seems the most likely. However, we cannot rule out some increase in receptor affinity. The overall results of either mechanism is a move to the right in the equation NA + adrenoceptor $\rightleftharpoons$ NA/adrenoceptor complex.

The increase in R_{max} was found to be significant only with pure β-lipoprotein. However, there was an upwards trend with the HC plasma. This increase must be associated with an increased intrinsic activity of the tissue and may be a result of tissue hyperexcitability or the recruitment of additional adrenoceptors. We consider either of these explanations possible.

These results would, therefore, indicate that when vascular muscle is exposed to cholesterol at a high concentration the muscle becomes more sensitive to NA. This hypersensitivity may be mediated (at least in part) by an inhibition of extraneuronal uptake mechanisms, similar to that previously reported in heart muscle.

We would like to thank Professor Mendelsohn, Dr E. Stein and his staff for collection of the plasma and cholesterol estimations. Also, Dr C. Altbrecht of the Council for Scientific and Industrial Research for assistance with the lipoprotein preparation. Reprint requests to T.A. McC.

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(Received March 31, 1975.)