LIPOPROTEINS AS A FACTOR REGULATING VASCULAR TONE

Yu. P. Vedernikov, V. Z. Lankin
A. K. Tikhaze, and A. M. Vikhert*

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Lipoproteins (LP) and, in particular, low-density LP (LDL), are regarded as an atherogenic factor. However, it is not known whether they have a direct action on vascular smooth muscles. It has been shown that the principal LP fractions induce relaxation of isolated vessels [2] and that LDL inhibit endothelium-dependent vascular relaxation [3, 4].

The aim of this investigation was to study the effect of the principal LP fractions — very low density LP (VLDL), LDL, and high density LP (HDL) — on isolated ring segments of the canine coronary artery and the rabbit thoracic aorta.

EXPERIMENTAL METHOD

LP were isolated from fresh blood plasma by the method in [5]. Isolated segments of the circumflex coronary of a dog and the thoracic aorta of a rabbit (5 mm wide) with intact endothelium or after its mechanical removal, were placed in a constant temperature chamber with a capacity of 10 ml, filled with Krebs–Henseleit solution (in mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.16, KH_2PO_4 1.18, $NaHCO_3$ 24.88, Na-EDTA 0.026, Na-pyruvate 2, glucose 11 (saturated with carbogen, 37°C, pH 7.35-7.4). During the stabilization period (60-90 min) a passive load was applied (20 mN). Changes in tension were recorded by UC-2 transducer on an R-711 dynograph ("Beckman," USA). To investigate the relaxing action of LP, the tone of the isolated segments of the coronary vessels was increased with potassium chloride (30 mM), and the tone of segments of the rabbit thoracic aorta by phenylephrine (10^{-6} M). Removal of the endothelium was verified by absence of relaxation in response to acetylcholine (10^{-6} M). The results are presented in the form of mean values and error of the mean.

EXPERIMENTAL RESULTS

Reduction of tension of the isolated segments of the canine coronary artery, induced by potassium chloride, through the action of various fractions of canine LP is illustrated in Fig. 1a. LP of all three classes were approximately equal in their ability to reduce tension of the coronary arterial segments, whether the endothelium was intact or removed. Changes in tension of segments of the rabbit thoracic aorta, whose tone was increased by phenylephrine, are shown in Fig. 1b. Canine LDL and HDL were equally effective in reducing tension of segments whether the endothelium was intact or removed. The weaker action of canine LP on the rabbit thoracic aorta will be noted (Fig. 1a and b). The ability of canine VLDL to induce relaxation of segments of the rabbit thoracic aorta was reduced 3 days after isolation of the segments to 10% of the phenylephrine contracture, compared with an 85% reduction of contracture on the day of isolation, with VLDL in the maximal concentration of 0.3 mg protein/ml. Canine VLDL activity on segments of the canine coronary artery persisted for a long time (Fig. 1a). Evidently LP do not exhibit high species specificity, for they induce relaxation of vessels of different species of animals [2], and the intensity of their effects differs purely quantitatively.

Original traces of experiments to study the effect of hog LP on isolated segments of the human and canine coronary artery are given in Fig. 2. A segment of the atherosclerotic human coronary artery, in the absence of endothelially-induced relaxation to the calcium ionophore

*Corresponding Member, Academy of Medical Sciences of the USSR.

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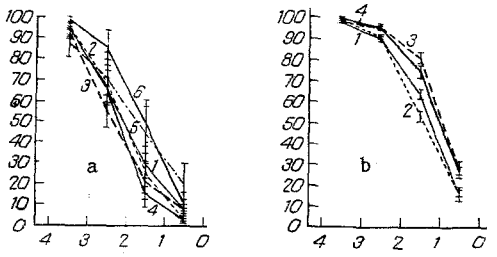

Fig. 1. Effect of canine LP on tension of isolated segments of canine coronary artery (a) and of rabbit thoracic aorta (b). a: 1) VLDL, E+ (n = 6); 2) VLDL, E– (n = 6); 3) LDL, E+ (n = 9); 4) LDL, E– (n = 8); 5) HDL, E+ (n = 5); 6) HDL, E– (n = 5); b: 1) LDL, E+ (n = 9); 2) LDL, E– (n = 10); 3) HDL, E+ (n = 9); 4) HDL, E– (n = 8). Abscissa, lipoprotein concentration ($-\log_{10}$), mg protein/ml; ordinate, contracture (in %) induced by calcium chloride (a; 30 mM) and phenylephrine (b; 10^{-6} M). E+) Endothelium intact, E–) endothelium removed.

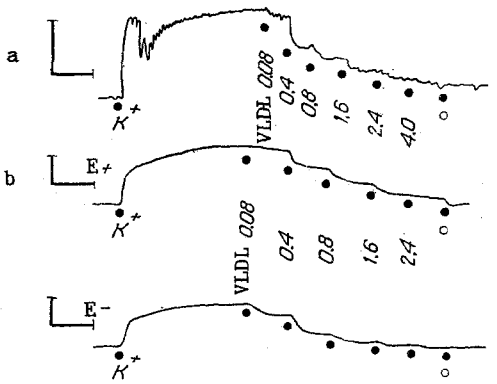

Fig. 2. Effect of hog VLDL on isolated atherosclerotic segment of human circumflex coronary artery (a) and segment of atherosclerotic canine coronary artery (b) with (E+) or after removal of (E–) endothelium. Calibration: vertical – 20 mN, horizontal – 10 min. Filled circles indicate addition of calcium chloride (K^+, 30 mM) and VLDL (in mg protein/ml). Empty circles – rinsing with Krebs–Henseleit solution.

A-23187, to acetylcholine (10^{-7} M), and to substance P (10^{-8} M), is relaxed by the action of hog VLDL (Fig. 2a). Segments of the canine coronary artery with the endothelium intact (E+) and removed (E–) responded by relaxation to hog VLDL (Fig. 2b). As Fig. 2 shows, a relaxation response was observed to sufficiently high VLDL concentrations.

No difference in the action of LP depending on the presence or absence of endothelium is visible in the illustrations shown. However, stronger relaxation of segments of arteries with intact endothelium could be observed in individual experiments. In some experiments using human LDL, distinct endothelially-dependent relaxation could be observed in segments of the rabbit thoracic aorta. Human LP evidently differ in this feature of their action from animal LP.

In similar experiments [3, 4] to study the effect of LDL and HDL (exposure for 30 min, followed by rinsing) a not very strong degree of endothelially-dependent relaxation of segments of the rabbit thoracic aorta in response to acetylcholine (10^{-9}-10^{-6} M) was observed. If segments of the aorta continued to be in contact with LP, inhibition of endothelially-dependent relaxation was substantial. In the last experiments the rate of development and the amplitude of tension induced by phenylephrine (10^{-6} M) were reduced, and its concentration had to be increased four- to sixfold for something approaching the control amplitude of contraction to be reached. Rinsing out the LP restored the effect of phenylephrine.

The ability of LP to relax isolated blood vessel preparations suggests that in vivo they may be a factor involved in the regulation of vascular tone. It is impossible, on the basis of these results, to stipulate high receptor and species specificity of action of LP, although the action of LDL on endothelially-dependent relaxation has been regarded as receptor-specific [3, 4].

LP not only have a direct relaxing action on vascular smooth muscles, but they can also inhibit endothelially-dependent relaxation, as is shown by weakening of acetylcholine-induced endothelially-dependent relaxation [3, 4]. Human LDL also induce endothelially-dependent relaxation. The endothelially-dependent effects of LP require more detailed study. The mechanism of the direct effect of LP on vascular smooth muscles, namely their ability to induce relaxation, is as follows. Having become incorporated into the membrane of vascular smooth-muscle cells, LP exert an influence on a certain stage of electromechanical coupling. They may perhaps reduce activation of voltage-controlled chemoreceptor calcium channels, thereby preventing the entry of extracellular calcium and of calcium released from intracellular pools. Support for this view is given by the ability of LP to relax tension induced by a hyperpotassium solution, and also depression of the rate of development and amplitude of contraction induced by phenylephrine. Data on the inhibitory effect of LP on cardiomyocyte membrane excitability confirm this hypothesis, for removal of LP from the culture medium of neonatal rat heart cells [7] or chick embryonic cells [6, 8] was accompanied by an increase in membrane excitability and an increase in sensitivity to tetrodotoxin and carbamoylcholine.

The function of LP as normal blood components is evidently to maintain low membrane excitability of vascular smooth-muscle cells by counteracting factors inducing their activation.

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