Communications to the Editor

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THROMBIN-INDUCED ENDOTHELIUM-DEPENDENT RELAXATION AND ITS INHIBITION BY LDL IN PORCINE CORONARY ARTERIES

Yasuhide Inoue, Takako Tomita*, Kazuki Nakamura, Masanori Ezaki, Koichi Nakayama
Department of Pharmacology, University of Shizuoka School of Pharmaceutical Sciences, Oshika, Shizuoka, 422, Japan

The effects of thrombin on the vascular tone of porcine coronary arteries and the influence of LDL on the thrombin effects were investigated. A segment of porcine coronary arteries was mounted under 1.5 g resting tension to measure the isometric force. LDL was prepared from porcine serum by ultracentrifugation and dialysis against Tyrode buffer. Thrombin (0.1-0.3 U/ml) dose-dependently relaxed intact porcine coronary arteries precontracted with $PGF_{2}\alpha$ (10^{-5} M). The removal of the endothelium by rubbing abolished the thrombin effect. This relaxation was also abolished by methylene blue (10^{-5} M), but was unaffected by indomethacin (5×10^{-5} M). At these concentrations, thrombin per se exerted a slight contractile effect on the resting tension of the coronary arteries with or without the endothelium. An addition of LDL (1 mg protein /ml) to the solution did not affect the precontraction of $PGF_{2}\alpha$. However, LDL dose-dependently inhibited the thrombin-induced relaxation of coronary arteries precontracted with $PGF_{2}\alpha$. Albumin (1 mg protein/ml) lacked such effects. Relaxation by sodium nitroprusside (10^{-6} M) was unaffected by the LDL-treatment. These results suggest that LDL, a major atherogenic factor, directly influences the endothelium-dependent vasodilation by thrombin in the coronary arteries.

KEYWORDS —— coronary artery; LDL; relaxation; thrombin; endothelium; pig

The importance of the intimal endothelium on vascular tone has drawn attention since the original observation of endothelium-dependent relaxation of the arteries by Furchgott and Zawadzki,[1] and was reinforced by the recent demonstration of the endothelium-derived constricting factor by Yanagisawa et al.[2] Several lines of evidence have suggested dysfunctions of the endothelium in atherosclerosis[3] and hypertension.[4] Predisposition to athelosclerosis, especially in the coronary arteries has been shown to be closely related to a high level of plasma low-density lipoprotein superimposed by hypertension.[5] In the present study, the vasoactive effects of thrombin, which have long been known to play an important role in the regulation of blood hemostasis and thrombosis,[6] and the influence of LDL on them were investigated in porcine coronary arteries. We found that thrombin induced endothelium-dependent relaxation, and the presence of LDL inhibited the thrombin effect.

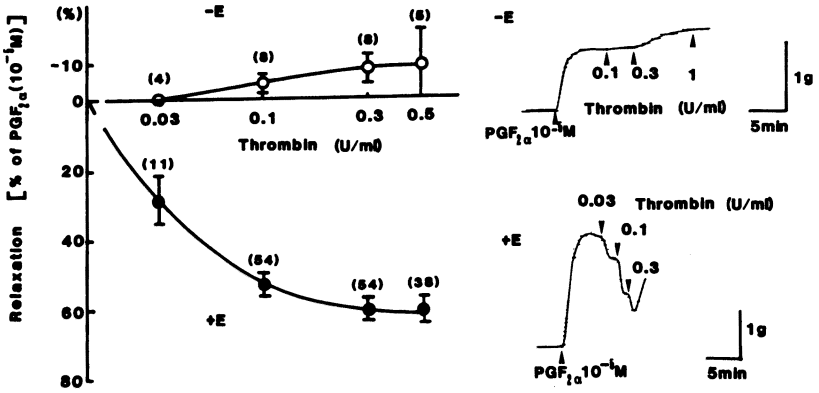

Fig. 1. Concentration-response curves for thrombin in the porcine coronary arteries with and without endothelium.
(Left) Strips of porcine coronary arteries with the endothelium (—●—, +E) and without (—○—,-E) were mounted in an organ bath containing Tyrode solution (pH 7.35) bubbled with 95% O_2-5% CO_2, and maintained at 37 °C. The tissues were precontracted with $PGF_2\alpha$ (10^{-5} M) and then stimulated with cumulative concentrations of thrombin. Data are expressed as percentage of the contractile responses to 10^{-5} M $PGF_2\alpha$. Each point and vertical bar represent mean ± S.E. for 4 to 54 different preparations.
(Right) Typical tracings of thrombin-induced relaxation and constriction in $PGF_2\alpha$ -precontracted arteries.

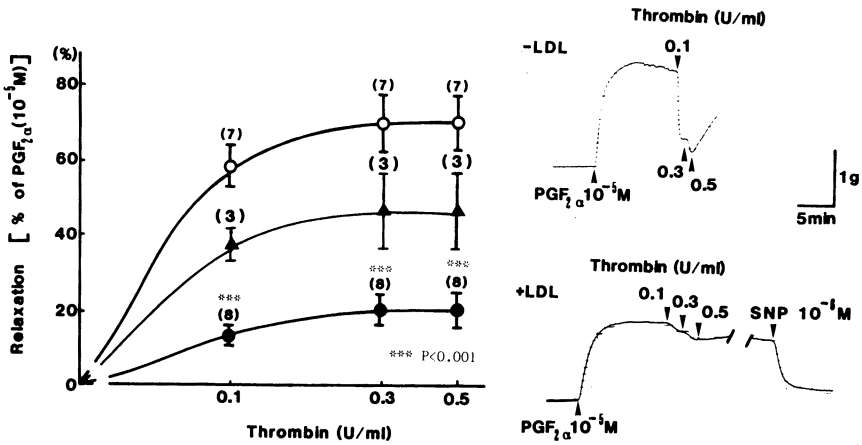

Fig. 2. Inhibitory effects of low-density lipoprotein (LDL) on thrombin-induced endothelium-dependent relaxation in pig coronary arteries.
(Left) Strips of pig coronary arteries were preincubated with LDL (—▲— : 0.5 mg, —●— : 1 mg protein/ml) or without(—○—) for 60 min in an organ bath containing Tyrode solution. LDL was prepared from pig serum by discontinuous density gradient ultracentrifugation and by dialysis against Tyrode solution. $PGF_2\alpha$ - precontracted tissues after the LDL-treatment, were relaxed by cumulative concentrations of thrombin. Each point and vertical bar indicate the mean ± S.E.. The number of experiments is indicated in the figures. *** Significant $P < 0.001$ (Student's t test)
(Right) Typical tracings of LDL effects on thrombin-induced endothelium-dependent relaxation. SNP: sodium nitroprusside

METHODS

Left anterior descending coronary arteries (approx. 3 mm O.D.) were rapidly isolated from the hearts of adult pigs of either sex weighing approx. 120 kg, and placed in Tris-Tyrode solution (pH 7.35, 30°C) gassed with 100% O_2. A 2-mm wide ring segment was cut open parallel to the longitudinal axis of the ring to form a strip. The strip was mounted vertically under 1.5 g of resting tension in organ baths filled with normal Tyrode solution (mM: NaCl 158.3, KCl 4, $CaCl_2$ 2, $NaHCO_3$ 10, NaH_2PO_4 0.42, glucose 5.6; pH 7.35; 37°C) and bubbled with a gas mixture of 95% O_2-5% CO_2. For endothelium-denuded preparations, the inner surface of the arteries was gently rubbed by a moistened cotton pledget. The removal of the endothelium was confirmed by the disappearance of relaxation with substance P (10^{-7} M). Isometric tension was measured by a force-displacement transducer (Nihon Kohden) and traced on a pen-writing recorder (Rikadenki R-G2). Preparations were allowed to equilibrate for at least 2 h before the actual experiments were started. LDL was prepared from porcine serum (density adjusted to 1.063 g/ml with KBr) by discontinuous density-gradient ultracentrifugation (100,000 x g for 20 h) and the collected LDL fraction was dialyzed for 24 h against three changes of normal Tyrode solution at 4°C.

Indomethacin, methylene blue, sodium nitroprusside and substance P were obtained from Sigma (St.Louis, Mo, USA), $PGF_2\alpha$ was supplied by Ono Pharmacetutical Co. (Osaka, Japan). Bovine albumin was from Armour (Kankakee, Ill, USA), and human thrombin from Midori Cross (Osaka, Japan).

RESULTS

$PGF_2\alpha$ (10^{-5} M) contracted porcine coronary arteries similarly with or without the endothelium [mean ± S.E. (n): 1.5 ± 0.3 g (5) and 1.6 ± 0.6 g (5) for isometric tension of preparations with and without the endothelium, respectively]. In intact arteries, human thrombin had no apparent effect on the basal tension at less than 0.30 U/ml, but induced some contraction at 0.5 U/ml, i.e., approx. 5% of contraction produced by $PGF_2\alpha$ (10^{-5} M). In endothelium-denuded arteries, the contractile response to thrombin was enhanced compared with the intact arteries (approx. 130% at 0.3 U/ml and 230% at 0.5 U/ml). However, addition of thrombin to intact arteries precontracted with $PGF_2\alpha$ (10^{-5} M) induced marked vasodilation dose-dependently. The vasodilation was evident at 0.03 U/ml of thrombin and reached a maximum at 0.3 U/ml (approx. 60%). This thrombin effect reached a maximum about 30 sec after administration (Fig. 1). In contrast, the removal of the endothelium by mechanical rubbing resulted in a complete loss of the thrombin-induced vasodilation; instead, there was a slight contractile response to thrombin (Fig. 1). The vasodilatory response to thrombin in intact arteries was not blocked by pretreatment for 40 min with indomethacin (5×10^{-6} M), but it was completely inhibited by methylene blue (10^{-5} M) for 60 min. The contraction produced by $PGF_2\alpha$ (10^{-5} M) became significantly greater with methylene blue treatment. Treatment of the coronary artery with LDL (1 mg/ml) from porcine serum for 60 min, influenced neither the resting tension nor the contraction by $PGF_2\alpha$ (10^{-5} M). But the LDL pretreatment (0.5 mg and 1 mg/ml, for 60 min) dose-dependently inhibited the thrombin-induced endothelium-dependent relaxation (Fig. 2). The relaxation by sodium nitroprusside (10^{-6} M) was remained after the LDL-treatment. Pretreatment with bovine serum albumin (1 mg/ml, 60 min) failed to inhibit the thrombin-induced endothelium-dependent relaxation.

DISCUSSION

Specific receptors with high affinity for thrombin, a key factor for normal hemostasis and thrombosis, are present in cultured human endothelial cells.[7] Our study showed that a small dose of thrombin induced endothelium-dependent vasodilation in porcine coronary arteries precontracted by $PGF_2\alpha$. The vasodilation was not affected by indomethacin which is a potent cyclo-oxygenase inhibitor, but it was completely blocked by methylene blue which inhibits soluble guanylate cyclase. These results suggest that thrombin-induced vasodilation is mediated by the formation of c-GMP and not by prostacyclin. This observation on thrombin-induced vasodilation of porcine coronary arteries is consistent with the reports in canine coronary arteries by Ku[8] and in canine femoral arteries by De Mey et al.[9]

Prior treatment of porcine coronary arteries with LDL at physiological concentration greatly diminished vasodilation due to thrombin. But the vasodilatory effect of sodium nitroprusside remained unchanged. This indicates that LDL specifically affected endothelium-dependent vasodilation. A similar inhibitory effect of LDL on endothelium-dependent relaxation by acetylcholine has been reported recently in rabbit aorta by Andrews et al.[10] It is not known whether the LDL inhibition shown by us is similar to that observed by Andrews et al.[10] LDL, whose level is positively correlated with the incidence of coronary heart diseases, influences arterial functions through several mechanisms. It also accelerates platelet functions due to changes in the membrane fluidity. Our study implies that beside such chronic effects, LDL may play a crucial role in acute regulation of the vascular tone in the coronary arteries.

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