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HEPARIN BINDING TO ENDOTHELIAL CELLS POTENTIATES THROMBIN-STIMULATED PGI_2 PRODUCTION. **T. Bârzu**[1], **P. Molho**[2], **R. Cariou**[2], **G. Tobelem**[2] and **J. Caen**[2]. Institut Choay[1], and INSERM U.150, Hôpital Lariboisière[2], Paris, France.

We studied the consequences of heparin (H) binding to endothelial cells (EC) on their basal and thrombin-stimulated PGI_2 production. Primary cultures of human umbilical vein EC were incubated with different H concentrations, in serum free medium for 5 hrs. The amount of 6-keto-$PGF_{1\alpha}$ was measured in the medium after 5 hrs with an enzyme-linked immunoassay. At all concentrations used (0.75 to 75 µg/ml) H did not alter the 5-hour basal production of PGI_2 (control, 10.1 ± 1.4 ng/10^6 cells; H 75 µg/ml; 10.8 ± 2.7 ng/10^6 cells). Basal or thrombin (0.1 U/ml)-stimulated (10 min) PGI_2 production was then determined using EC bearing only bound heparin. The low, unstimulated PGI_2 release (0.412 ± 0.04 ng/10^6 cells) was not significantly changed in the presence of bound H, but the thrombin-stimulated release was potentiated.

Table : Effect of bound heparin on thrombin-induced PGI_2 production (6-keto $PGF_{1\alpha}$, ng/10^6 cells)

Without H	In the presence of bound H after incubation with different H concentrations (µg/ml)			
	0.75	3.0	15.0	75.0
15.2 ± 1.7	46.5 ± 9.6	55.3 ± 6.4	51.8 ± 6.4	56.8 ± 9.4

The K_D for H binding to EC is 2.5 µg/ml. Thus at 3 µg/ml, half maximal saturation of binding sites and maximal potentiation of thrombin action were achieved. This concentration of bound H shifted the dose-response curve of thrombin induced PGI_2 production to the left. Similar effect was obtained with half maximal saturating concentration of low molecular weight H (CY 222). Neither arachinodate nor LC4-induced PGI_2 release were modified by H binding to EC, suggesting that potentiation is specific to thrombin. Since bound H was shown to not modify the high affinity thrombin binding to EC, the potentiating effect of bound H could related rather to interference with the specific mechanism of thrombin-stimulation of PGI_2 production.

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MECHANISMS INVOLVED IN 5-HT STIMULATION OF PROSTACYCLIN PRODUCTION BY BOVINE AORTIC SMOOTH MUSCLE CELLS. D. Demolle, E.J. Cragoe* and J.M. Boeynaems. Institute of Interdisciplinary Research, School of Medicine, Free University of Brussels, Brussels, Belgium; *Merck Sharp Dohme Research Laboratories, West-Point, USA.

Serotonin (5-HT) stimulates prostacyclin (PGI_2) production by bovine aortic smooth muscle cells in culture via $5\text{-}HT_2$ receptors (1). These cells express a synthetic phenotype (2), whereas the majority of the smooth muscle cells in the media from adult arteries are in a contractile state. We have now shown that 5-HT (1-10 µM) also stimulates PGI_2 production by a preparation of contractile smooth muscle cells : explants from bovine aortic media cultured for short periods. This effect is independent from $5\text{-}HT_2$ receptors : it is only partially inhibited (±30%) by ketanserin (a selective and potent $5\text{-}HT_2$ antagonist) and is perfectly mimicked by a $5\text{-}HT_1$ agonist, 5-carboxamidotryptamine. $5\text{-}HT_2$ receptors seem to be linked to a phospholipase C (3), with subsequent accumulation of inositol trisphosphate, $Ins(1,4,5)P_3$, and diacylglycerol, an activator of protein kinase C. We have observed a stimulatory effect of phorbol 12-myristate, 13-acetate (a selective activator of kinase C) on PGI_2 production by the bovine aortic smooth muscle cells (synthetic state), whereas it was totally ineffective on media explants preparation (contractile state). Furthermore, in the smooth muscle cells in culture, the 5-HT effect can be inhibited by (ethyl-isopropyl)amiloride, a potent and selective inhibitor of the Na^+/H^+ antiporter. In conclusion it appears that the regulation mechanisms of PGI_2 production in arterial smooth muscle cells are strongly dependent on the phenotypic state of these cells. The control of PGI_2 release via $5\text{-}HT_2$ receptors seems to involve a cytoplasmic alkalinization, via the activation of protein kinase C. The mechanism of 5-HT action in the media explants remains to be elucidated.

(1) Coughlin, S.R. et al.: Proc. Natl. Acad. Sci. USA 78(11), 7134-7138, 1981.
(2) Chamley-Campbell, J.H. and Campbell, G.K.: Atherosclerosis 40, 347-357, 1981.
(3) Roth, B.L. et al.: J. Pharm. Exp. Ther. 238(2), 480-485, 1986.

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ACUTE THROMBOSIS IN STENOTIC AREAS: IMPORTANCE OF THE VASCULAR MATRIX EXPOSED TO BLOOD. L. Badimon, J. J. Badimon and V. Fuster. Division of Cardiology, The Mount Sinai Medical Center, N. Y.

The platelet response to angioplasty or spontaneous plaque rupture leads to acute thrombotic occlusion under certain conditions. We analyzed the role of local shear rate (flow and vessel cross-section related), the nature of the exposed matrix and the effect of thrombin inhibition in platelet acute response to injury. Collagen type I (exposed in plaque rupture) and de-endothelialized pig aorta (mild injury) were exposed to pig blood in a tubular perfusion chamber with well characterized flow conditions, placed within an extracorporeal circuit in swine (N=20). Platelet deposition was measured by labeling autologous platelets with ^{111}Indium and optical morphometry of epoxy embedded specimens. Selected specimens were analyzed by electron microscopy. Unanticoagulated blood and blood from animals treated with 300u/Kg of heparin were perfused over the substrate for 3 and 10 min at local shear rates typical of unobstructed arteries ($212s^{-1}$ - $424s^{-1}$) and of stenotic vessel ($824s^{-1}$ - $1690s^{-1}$). Platelet deposition (Platelets x $10^6/cm^2$ ±SE) for 3 min perfusions were:

	Heparin	Unobstructed	Stenotic
Aorta	+	5 ± 0.7	16 ± 3
Collagen	+	29 ± 7	165 ± 37
Aorta	-	2.5 ± 0.4	20 ± 6
Collagen	-	45 ± 12	255 ± 30

Platelet deposition is dependent on the reactivity of the vascular matrix exposed to blood and on the local shear rate. The greatest rate of thrombus growth is observed with collagen and high shear rate conditions which may precipitate acute thrombotic occlusion in stenotic regions, mainly when the coagulation pathway is not inhibited ($255x10^6$ platelets/ cm^2 in 3 minutes. The relative contribution of rheology and the isolated components of the atherosclerotic plaque matrix exposed to blood in the onset of acute coronary syndromes will be differentiated with this experimental model.

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PLATELET ALTERATIONS IN RESPONSE TO REPETITIVE, SHORT-DURATION LAMINAR SHEAR STRESS. J.H. Joist (1), J.E. Bauman (1), and S.P. Sutera (2). Departments of Internal Medicine and Pathology, St. Louis University (1), and Department of Mechanical Engineering, Washington University (2), St. Louis, MO, USA.

We examined platelet aggregation (PAG = loss of single platelets), platelet dense granule release, and platelet injury (LDH loss) in normal human citrated platelet-rich plasma subjected to biologically more relevant repetitive, laminar shear stress of 25 and 50 dyn/cm^2 in a computer-controlled cone-plate viscometer. Shear pulse duration (1-3 sec), shear pulse ramp function (rate of shear stress increase and decrease per pulse, 0.6-4 sec), number of shear pulses (1-20) and pauses between shear pulses (0-5 sec) were varied in different combinations to assess the effects of each variable on platelet alterations. Maximum PAG (92±8%) was observed with three 1 sec shear pulses, 0.6 sec ramp function and 1 sec between shear pause. PAG decreased with increasing ramp function, increasing number of shear pulses (>10), and increasing pause duration. Rapid platelet deaggregation (starting at 5 sec) was observed after a single 1 sec shear exposure. The rate of deaggregation decreased with increasing shear pulse number, increasing shear pulse amplitude, and increasing shear pulse duration. In contrast to PAG, dense granule release increased progressively with increasing shear pulse number, duration, and amplitude. No appreciable platelet injury (LDH loss) was observed under the conditions used. The findings indicate that massive reversible PAG can be induced by a single 1 sec shear pulse and that the extent of PAG with more prolonged, repetitive shear exposure is largely a function of platelet deaggregation rather than PAG. Thus, data previously reported from our laboratory and other investigators using prolonged (>5 sec) exposure of platelets to shear stress may require reevaluation.