Biophysical Journal, Volume 98

Supporting Material

Single Protein Molecule Mapping with Magnetic Atomic Force Microscopy

Andriy V. Moskalenko, Polina L. Yarova, Sergey N. Gordeev, and Sergey V. Smirnov

Supplementary Material

1. Functional bioavailability of biotinylated ET-1 (bET-1): Intact artery study. Functional confirmation of bioavailability of bET-1 was investigated in intact rat small mesenteric arteries (3rd branch) using a small vessel wire myograph (model 400A, DMT, Aarhus, Denmark) to measure force of contraction as previously described (1,2). Briefly, a short segment (~2 mm) of a small mesenteric artery was mounted on the wire myograph. Tissue was maintained at 37°C in aerated Krebs solution of the following composition (in mM): NaCl 118, $NaHCO_3$ 25, KCl 3.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11 and $CaCl_2$ 2.5. Tissue viability was verified with 1-5 μM α-adrenoceptor agonist phenylephrine (PE) following by relaxation to 100-300 nM acetylcholine (ACh) (All from Sigma, Gillingham, UK) (Fig. S1***A***). Fig. S1***B*** demonstrates that 20 and 40 nM bET-1 causes the same contraction as 5 μM PE, which was used to elicit maximal contractile response in this artery. Note that contraction was maintained after wash-out (WO) of bET-1, demonstrating its slow reversibility due to tight binding to the receptors (3). The lack of reversibility of the effect of ET-1 following the wash-out of this vasoconstrictor prevents the direct comparison of bET-1 and non-biotinylated ET-1 in the same preparation. However, similar effects were previously demonstrated for non-modified ET-1 (4,5). Therefore, biotinylation of ET-1 did not significantly alter its ability to bind and activate the receptors.

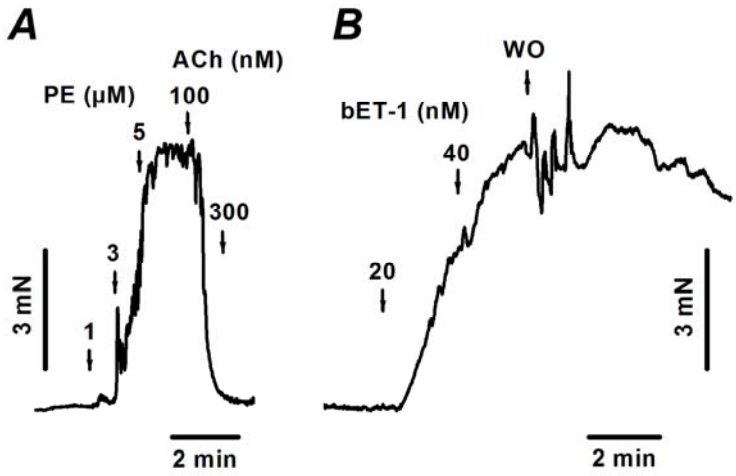

Figure S1. Functional bioavailability of bET-1. (***A***) Contraction of small mesenteric artery to 1, 3 and 5 μM of α-adrenoceptor agonist phenylephrine (PE). The contracted artery was subsequently relaxed with 100 and 300 nM acetylcholine (ACh), demonstrating that the artery is in good condition and has intact endothelium. (***B***) Contractile responses of the same artery to 20 and 40 nM biotinylated ET-1 (bET-1).

2. AFM and MFM imaging of control non-biotinylated smooth muscle cells.

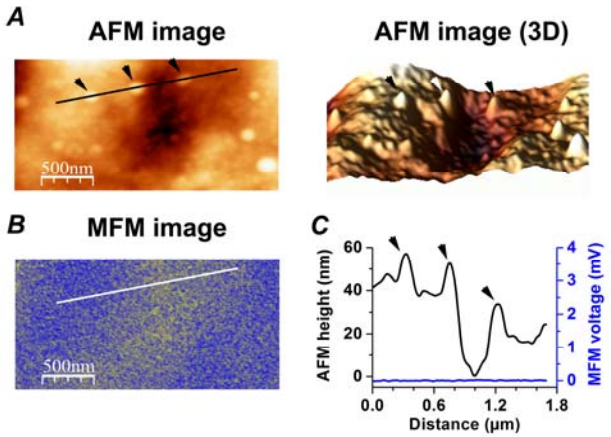

Figure S2. Comparison of AFM and MFM imaging of the surface of control *non-biotinylated* cultured aortic SMC. (***A***) AFM image in tapping mode (left) and its 3D reconstruction (right) of a 2.5 x 1.25 μm area of the surface of a non-biotinylated cultured SMC. (***B***) MFM image of ***A*** in the standard lift mode at 50 nm. (***C***) Line analysis of the same area in the AFM and MFM images (shown by straight lines in ***A*** and ***B***). Distinctive topographic features on the surface of the non-biotinylated cell present in the AFM image (shown by the black line), but absent in the MFM image (shown by the blue line). The position of arrowheads in ***C*** corresponds to the three distinctive AFM amplitude peaks shown highlighted by arrowheads in the AFM image and the 3D reconstruction image in ***A***. The mismatch between AFM and MFM images of control (non-biotinylated) cells demonstrates lack of non-specific binding between superparamagnetic microbeads and the cell surface. It also illustrates the advantage of the MFM imaging, which will recognize only magnetically labelled targets, compared to AFM imaging alone which will not discriminate between specific and non-specific typographical features on the cell surface.

3. Confocal imaging of primary aortic myocytes labelled with bET-1 and caveolin-1. Primary cultured aortic smooth muscle cells were fixed with 3% paraformaldehyde, then blocked with BSA and stained with b-ET-1 as descried in the Methods section of the paper. The bET-1 labelled cells were washed with phosphate buffered saline (PBS) and then permeabilized with 0.1%Triton and 10% BSA for 20 min in PBS. Cells were subsequently incubated with Anti-Caveolin-1, Cy3 Conjugate rabbit antibody (1:100 dilution) (Sigma, Gillingham, UK C3990) in 10% BSA in PBS overnight at room temperature using bellydancer. The incubation solution also contained anti-Biotin FITC conjugate (1:150) to label bET-1 attached to ET receptors. Confocal imaging was performed using Plan-Neofluar 40x/1.3 oil objective. FITC fluorescence was recorded using the Argon 488 nm excitation wavelength and emission bandwidth of 505-530 nm. Cy3 was collected using the HeNe laser 543 nm excitation wavelength and LP 585 nm filter.

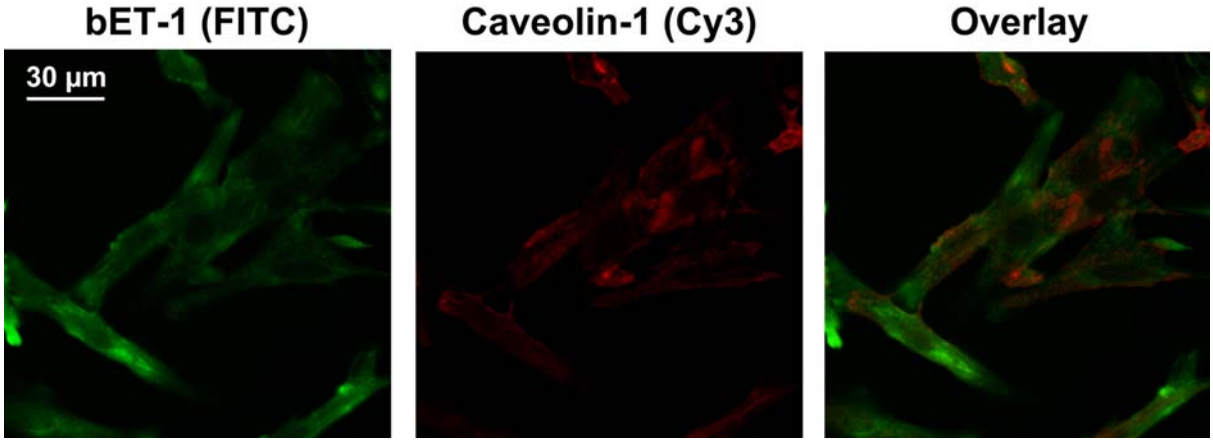

Figure S3. Lack of significant co-localization between ET receptors labelled with bET-1 and caveolin-1 in rat aortic smooth muscle cells. Primary cultured aortic smooth muscle cells were double labelled with bET-1 and FITC (green, left panel) and with anti-caveolin-1 Cy3 conjugate (red, middle panel). Overlay (right panel) show little overlap between the two fluorescent signals indicating the lack of significant co-localization of ET receptors and caveolin-1.

Supplementary References

1. Dora,K.A., J.M.Hinton, S.D.Walker, and C.J.Garland. 2000. An indirect influence of phenylephrine on the release of endothelium-derived vasodilators in rat small mesenteric artery. *Br. J. Pharmacol.* 129:381-387.
2. Vanková,M., V.A.Snetkov, G.A.Knock, P.I.Aaronson, and J.P.Ward. 2005. Euhydric hypercapnia increases vasoreactivity of rat pulmonary arteries via HCO_3 transport and depolarisation. *Cardiovasc. Res.* 65:505-512.
3. Rubanyi,G.M. and M.A.Polokoff. 1994. Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol. Rev.* 46:325-41
4. Itoh,H., H.Higuchi, N.Hiraoka, M.Ito, T.Konishi, T.Nakano, and K.Lederis. 1991. Contraction of rat thoracic aorta strips by endothelin-1 in the absence of extracellular Ca^{2+}. *Br. J. Pharmacol.* 104:847-852.
5. Salameh,G., M.R.Karamsetty, R.R.Warburton, J.R.Klinger, L.C.Ou, and N.S.Hill. 1999. Differences in acute hypoxic pulmonary vasoresponsiveness between rat strains: role of endothelium. *J. Appl. Physiol.* 87:356-362.