ELSEVIER

European Journal of Pharmacology 351 (1998) R1–R3

ejp

Rapid communication

Microtubule disruption potentiates phenylephrine-induced vasoconstriction in rat mesenteric arterial bed

Romulo Leite *, R. Clinton Webb

Department of Physiology, 7813 Medical Sciences Building II, University of Michigan Medical School, Ann Arbor, MI 48109-0622, USA

Received 6 May 1998; accepted 8 May 1998

Abstract

The pressor response induced by phenylephrine in the rat isolated mesenteric arterial bed was significantly increased following treatment with nocodazole, a drug that disassembles microtubules (10 μM, 90 min). This increase was even greater in the presence of nitric oxide (NO) synthase inhibition, and completely reversed by paclitaxel (20 μM), a stabilizer of microtubules. These results demonstrate that disassembly of microtubules enhances vasoconstriction to receptor activation, suggesting that the microtubules modulate the transduction of intracellular signals in endothelium and vascular smooth muscle cells.

Keywords: Microtubule; Blood vessel; Nocodazole

Cytoplasmic microtubules serve as a network by which vesicles and membrane-bound organelles can travel. In addition, microtubules regulate cell shape and movement and participate in several cell signaling events (PeMan et al., 1983; Rowinsky et al., 1990). Disruption of microtubules induces shortening in cultured cells and potentiates agonist-induced contraction in isolated pulmonary arteries from rats (Sheridan et al., 1996). In this study we tested the hypothesis that microtubules oppose contraction induced by receptor activation.

Mesenteric arterial beds from normotensive Sprague–Dawley rats were perfused at 2.5 ml/min with a modified Krebs solution (37°C and pH 7.4) and the perfusion pressure was continuously monitored. A dose-response curve to phenylephrine (1.56–400 nmol) was performed after a 30 min stabilization period, followed by incubation with either an inhibitor of microtubular assembly, nocodazale (10 μM), or vehicle (dimethyl sulfoxide, DMSO). After a 90 min incubation period another dose-response curve was again established. The same protocol was repeated in the presence of N^{ω}-nitro-L-arginine (L-NNA, 300 μM), an inhibitor of nitric oxide (NO) synthase, alone or combined with the microtubule stabilizer paclitaxel (20 μM).

The pressor response induced by phenylephrine was significantly potentiated in the presence of nocadazole (ED_{50} 101.3 vs. 79.8 nmol, Fig. 1A and D). Treatment with L-NNA caused a significant left shift of the dose–response curve induced by phenylephrine. The presence of nocodazole in the L-NNA treated vessels induced a further potentiation (ED_{50} 17.6 vs. 4.5 nmol, Fig. 1B and D). This effect induced by nocodazole was completely blocked by paclitaxel (ED_{50} 13.8 vs. 12.0 nmol, Fig. 1C and D).

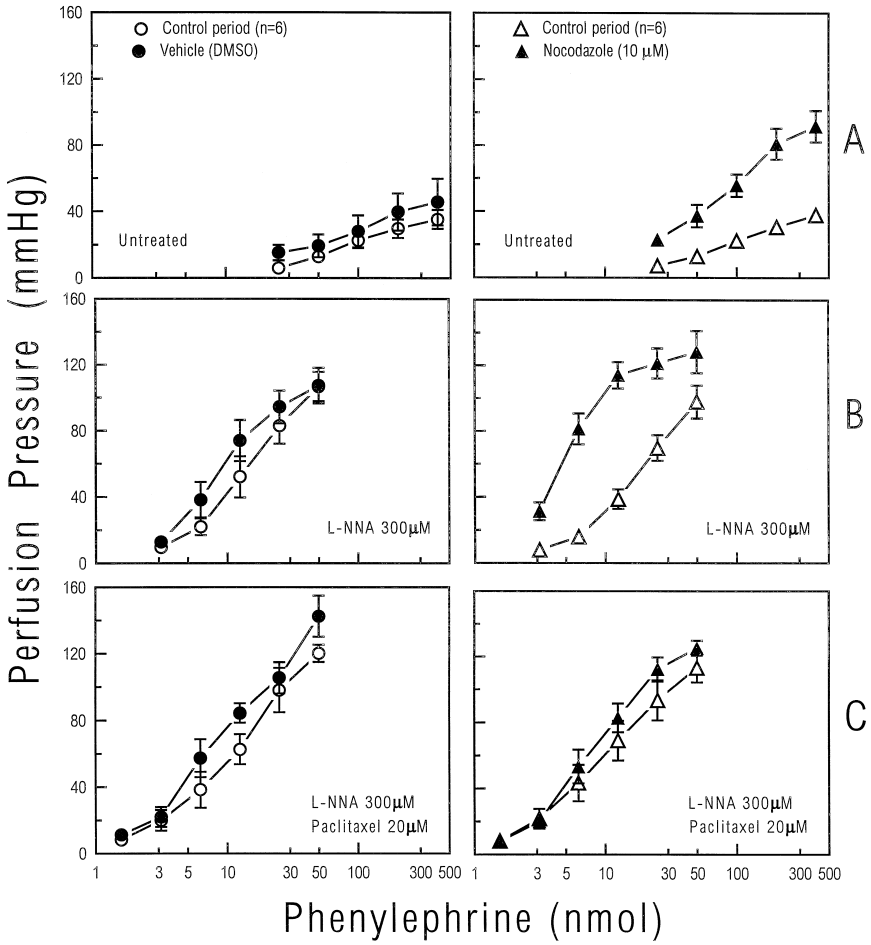
Phenylephrine elicits intracellular responses by activating receptors linked to G_{α}-mediated stimulation of phospholipase C which hydrolyzes the lipid precursor phosphatidyilinositol 4,5-biphosphate (PIP_2) to generate second messengers, diacylglycerol and inositol 1,4,5-triphosphate (IP_3). Diacylglycerol and IP_3 trigger a variety of cellular functions by activating protein kinase C and mobilizing stored calcium (Malarkey et al., 1996).

It has been suggested (Popova et al., 1997) that the activity of phospholipase C is regulated through the interaction of tubulin, a cytoskeletal protein that constitutes the microtubules, with a specific G protein α subunit or with the phospholipase C substrate PIP_2.

Kolodney and Elson (1995) have shown that disruption of microtubules increases phosphorylation of the myosin regulatory light chain (LC_{20}) in cultured fibroblasts. This effect could be explained by an increased activity of myosin light chain kinase or a reduced activity of myosin

* Corresponding author. Tel.: +1-734-763-5606; fax: +1-734-647-7950; e-mail: rlicbfar@mono.icb.ufmg.br

0014-2999/98/$19.00



D

TREATMENT		Control	Vehicle	Control	Nocodazole
UNTREATED	-logED50±SE	7.07±0.05	7.11±0.05	6.99±0.04	7.10±0.05 *
	ED50 (nmol)	84.9	78.2	101.3	79.8
L-NNA	-logED50±SE	7.97±0.09	8.17±0.11	7.76±0.03	8.31±0.08 *
	ED50 (nmol)	10.6	6.8	17.6	4.5
L-NNA PACLITAXEL	-logED50±SE	7.81±0.08	7.87±0.09	7.86±0.10	7.92±0.08
	ED50 (nmol)	15.6	13.6	13.8	12.0

Fig. 1. Dose–response curve induced by phenylephrine in rat isolated mesenteric arterial bed in the presence of vehicle (DMSO) and nocodazole (10 μM) in untreated (A), L-NNA (300 μM) treated (B) and L-NNA (300 μM) and paclitaxel (20 μM) treated vessels (C). Panel D shows the $-\log ED_{50} \pm$ S.E. and the ED_{50} (nmol) calculated for each curve showed in panels A, B, and C. ($n = 6$) represents the number of experiments for each different protocol. $* P < 0.05$ (paired Student t-test).

light chain phosphatase induced by an increased protein kinase C activity.

Thus, the potentiation of the pressor response induced by phenylephrine in the presence of nocodazole, a drug causing microtubular disassembly or inhibition of microtubule polymerization (Hoebeke et al., 1976), could be explained by an improvement of the phospholipase C signaling within the cell after disruption of microtubules, that could be causing an increase of the activity of the phospholipase C due to either a greater activation of G protein or an increase of PIP_2 availability.

We can also hypothesize that microtubule disruption activates the endothelial NO synthase, since the potentiation of the pressor response induced by phenylephrine by nocodazole in the presence of L-NNA, a NO synthase inhibitor, was much greater compared to that observed in untreated mesenteric vessels. This result supports the idea that disassembling microtubules would favor the disruption of the endothelial NO synthase-calveolin complex induced by agonist-promoted increases in $[Ca^{2+}]_i$ which leads to an increase of the enzyme activity and an increase of NO production (Feron et al., 1998).

The effect of nocodazole in these experiments appears to be specific since the antineoplastic compound paclitaxel (former generic name taxol), which is known to cause an increased assembly of stable microtubules (Rowinsky et

al., 1990), completely reverted the potentiation due to nocodazole.

These results demonstrate that disassembly of microtubules enhances vasoconstriction to receptor activation, suggesting that the microtubules modulate the transduction of intracellular signals in endothelium and vascular smooth muscle cells.

Acknowledgements

This study was supported by research grants and fellowships from the NIH (USA), Fubrigth Commission (USA) and CAPES (Brazil). Dr. Romulo Leite is a visiting researcher from the Department of Pharmacology, Federal University of Minas Gerais, Belo Horizonte, MG 31270-901, Brazil.

References

Feron, O., Saldana, F., Michel, J.B., Michel, T., 1998. The endothelial nitric-oxide synthase-calveolin regulatory cycle. J. Biol. Chem. 273, 3125–3128.

Hoebeke, J., Van Nijen, G., De Brabander, M., 1976. Interaction of oncodazole (R 17934), a new antitumoral drug, with rat brain tubulin. Biochem. Biophys. Res. Commun. 69, 319–324.

Kolodney, M.S., Elson, E.L., 1995. Contraction due to microtubule disruption is associated with increased phosphorylation of myosin regulatory light chain. Proc. Natl. Acad. Sci. USA 92, 10252–10256.

Malarkey, K., Aidulis, D., Belham, C.M., Graham, A., McLees, A., Paul, A., Plevin, R., 1996. Cell signalling pathways involved in the regulation of vascular smooth muscle contraction and relaxation. In: Garland, C.J., Angus, J.A. (Eds.), Pharmacology of Vascular Smooth Muscle. Oxford Univ. Press, New York, NY, pp. 160–183.

PeMan, S., Capco, D.G., Fey, E.G., Chatterjee, P., Reiter, T., Ermish, S., Wan, K., 1983. The three dimensional structural networks of cytoplasm and nucleus: functions in cells and tissue. Mod. Cell Biol. 2, 385–415.

Popova, J.S., Garrison, J.C., Rhee, S.G., Rasenick, M.M., 1997. Tubulin, G_q, and phosphatidylinositol 4,5-biphosphate interact to regulate phospholipase Cβ_1 signaling. J. Biol. Chem. 272, 6760–6765.

Rowinsky, E.K., Cazenave, L.A., Donehower, R.C., 1990. Taxol: A novel investigational antimicrotubule agent. J. Natl. Cancer Inst. 82, 1247–1259.

Sheridan, B.C., McIntyre, R.C. Jr., Meldrum, D.R., Cleveland, J.C. Jr., Agrafojo, J., Banerjee, A., Harken, A.H., Fullerton, D.A., 1996. Microtubules regulate pulmonary vascular smooth muscle contraction. J. Surg. Res. 62, 284–287.