Vol. 171, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1990 Pages 143-147

MECHANOSENSITIVE ADENYLATE CYCLASE ACTIVITY IN CORONARY VASCULAR SMOOTH MUSCLE CELLS

Ira Mills[1], George Letsou*, Joseph Rabban, Bauer Sumpio*, and Henry Gewirtz

Division of Cardiology, Brown Program in Medicine, Rhode Island Hospital, Providence, Rhode Island 02903

*Department of Surgery, Yale University School of Medicine, New Haven, CT 06510

Received July 12, 1990

SUMMARY: The purpose of the present study was to test the hypothesis that adenylate cyclase activity of porcine coronary artery smooth muscle cells is sensitive to mechanical stretch. Cultured vascular smooth muscle cells were stretched at 24% maximal strain at 60 cycles/min for 30 minutes. Both basal and maximal catalytic activity of adenylate cyclase (as assessed by stimulation by 100 uM forskolin with 5 mM manganese chloride) were reduced by 30% ($P<0.05$) in membranes obtained from stretch versus unstretched cells. The magnitude of the stretch-induced reduction in Gpp(NH)p was identical over the entire time course studied (5-30 minutes). Furthermore, basal adenylate cyclase activity was inversely related to the magnitude of stretch. Thus, cyclic stretch can influence adenylate cyclase activity in coronary vascular smooth muscle cells. These data provide important information concerning potential biochemical mechanisms involved in the myogenic response of vascular smooth muscle and also suggest a potential mechanism by which the coronary circulation may adapt to chronically reduced perfusion pressure.

Atherosclerosis of the human coronary circulation is a chronic disease which often is well tolerated for prolonged periods of time. Dilation of downstream arterioles is the principal compensatory response involved in minimizing the effects of proximal atherosclerotic narrowing of the coronary vasculature. The signaling mechanism involved, however, is unknown as is the precise mechanism by which arteriolar dilation is maintained. Since results from previous experiments conducted in our laboratory (1) indicate that the signal for maintenance of sustained arteriolar dilation probably is not a metabolic one, we tested the hypothesis that a mechanosensitive mechanism coupled to adenylate cyclase may be involved. If the hypothesis is correct, then we would expect increased stretch to be associated with reduced adenylate cyclase activity and vice versa (2). A reduction in adenylate cyclase activity would facilitate vasoconstriction by inactivation of the cyclic AMP second messenger pathway. Cyclic stretch of porcine coronary artery smooth muscle cells was employed in order to test this hypothesis.

[1] To whom correspondence should be addressed.

Abbreviation used: Gpp(NH)p,5'-guanylyl-imidodiphosphate.

0006-291X/90 $1.50

MATERIALS AND METHODS

Porcine Coronary Artery Smooth Muscle Cell Culture: Cultured vascular smooth muscle cells were prepared as described by Gunther et al. (3). Coronary arteries were obtained from pigs freshly killed either at a local slaughterhouse or in the laboratory. Adventitia was dissected away and the endothelium scraped off with a scalpel blade. The vessels were cut into small pieces and digested in DMEM containing 2 mg/ml collagenase, 0.250 mg/ml elastase and 0.5% fetal calf serum. Tissue digestion was carried out in a gently shaking water bath for 90 minutes at 37°C. Harvested cells were resuspended in DMEM containing 10% fetal calf serum, 1% penicillin/streptomycin and 1% glutamine. Cells were fed every 24-48 hours and reached confluence after 7-12 days. Cells were used between the second and tenth passages.

Cells were harvested and membranes prepared as described previously with minor modification (4). At confluence, the cells were rinsed 2 times with PBS and scraped with a rubber policeman in 10 mls of PBS. The cells were centrifuged at 400 x g for 5 minutes and resuspended in homogenization buffer containing 10 mM Tris, 1 mM EDTA, and 1 mM DTT, pH 7.5. The cells were homogenized in a Type A Dounce homogenizer for 10 strokes. The homogenate was centrifuged at 27000 x g for 30 minutes. The pellet obtained was used to assay adenylate cyclase activity. Protein was determined according to Lowry (5).

Vacuum operated stretch of cultured coronary vascular smooth muscle cells: Coronary vascular smooth muscle cells were plated (300,000 cells per well) onto six-well collagen coated plates with flexible bottoms. The cells were allowed to attach for 2 days prior to stretch. The stretching regimen consisted of 24% maximal elongation at 60 cycles per minute for 0,5,10,15 and 30 minutes. The stretch apparatus (Flexercell Strain Unit, McKeesport, PA 15132) consists of a vacuum unit, a solenoid valve and is controlled by a computer with a timer program. Culture wells were deformed to a known percent elongation by application of a precise vacuum. The stretch was translated to the adherent vascular smooth muscle cells. Real-time video experiments demonstrated that vascular smooth muscle cells remain attached to the well during the stretch (unpublished observations). Upon release of the vacuum, culture wells return to their original conformation. Force analysis of the strain on the flexible well during stretch at various vacuum levels (i.e., increasing levels of deformation) has been calculated mathematically, by finite element analysis (6) and empirically by measuring with a micrometer the distance between concentric circles (radial strain) or diametric axes (axial strain) marked on the membrane. Very little change is observed in the latter, hence the force on the attached cells is uniaxial.

Measurement of adenylate cyclase activity: Adenylate cyclase activity was measured as described previously (7). The incubation buffer contained 200 uM alpha-[^{32}P]ATP (50 cpm/pmol), 30 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 0.1 mM cyclic AMP, 0.1% bovine serum albumin, 10 mM creatine phosphate, 10 U/ml creatine phosphokinase, 1 mM DTT, and the indicated additions (vide infra) in a final volume of 100 ul. Assays were initiated by the addition of membranes (20 ug) and were permitted to run for 15 minutes at 37° C in a shaking water bath. The assay was terminated by the addition of 1% SDS. Cyclic [^{32}P]AMP was purified by sequential chromatography on Dowex and alumina columns (8). Values of adenylate cyclase activity (pmoles/min/mg protein) were corrected for incomplete recovery. Cyclic [^{3}H] AMP was added as a recovery standard. The recovery of cyclic AMP after elution of Dowex and alumina columns was 65%.

Statistical analysis: Data were analyzed by paired t-test. All results are expressed as mean $\pm$ SEM. Statistical significance was set at $P<0.05$.

Chemicals: Forskolin was purchased from Calbiochem. Gpp(NH)p was purchased from Boehringer Mannheim. [^{32}P]ATP and [^{3}H]cyclic AMP were purchased from New England Nuclear. All other reagents were purchased from Sigma.

RESULTS AND DISCUSSION

Basal adenylate cyclase activity was affected by the magnitude of cell stretch. A pressure of 20 kPascals (Figure 1) was required to produce a statistically significant effect

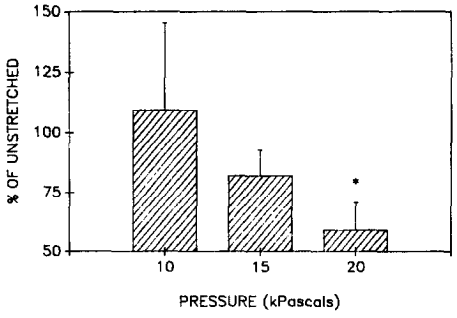


Figure 1. Effect of the magnitude of stretch on basal adenylate cyclase activity in cultured coronary vascular smooth muscle cells. The values represent the mean ± SEM; N=4. *=p<0.05 (as compared to unstretched control by paired t-test).

although a trend in the direction of reduced adenylate cyclase activity was seen at 15 KPascals. Basal and maximally stimulated adenylate cyclase activity (100 uM forskolin with 5 mM $MgCl_2$) were reduced significantly in membranes obtained from cultured coronary vascular smooth muscle cells stretched for 30 minutes as compared to unstretched cells (Figures 2A and 2B). As shown in Figure 3, Gpp(NH)p (10^{-7}-10^{-5}M) stimulation of adenylate cyclase also was reduced in membranes obtained from stretched versus unstretched cells. The stretch-induced reduction in 0.1 uM Gpp(NH)p stimulated enzyme activity was maximal at 5 minutes and not enhanced by longer periods of stretch up to 30 minutes (Figure 4). A similar trend was observed at concentrations of GPP(NH)p and forskolin of 1.0, 10, and 100 μM (data not shown).

The data obtained in the present study supports the hypothesis that adenylate cyclase activity of porcine coronary vascular smooth muscle cells is sensitive to cyclic tensional deformation in culture. Adenylate cyclase activity has been shown to be sensitive to both acute and chronic mechanical stimulation in other tissues. Acute stretch of cultured osteocytes caused an increase in adenylate cyclase activity (9). Perfusion of rat hearts for 1 hour at elevated aortic pressures caused an increase in cyclic AMP levels and stimulated

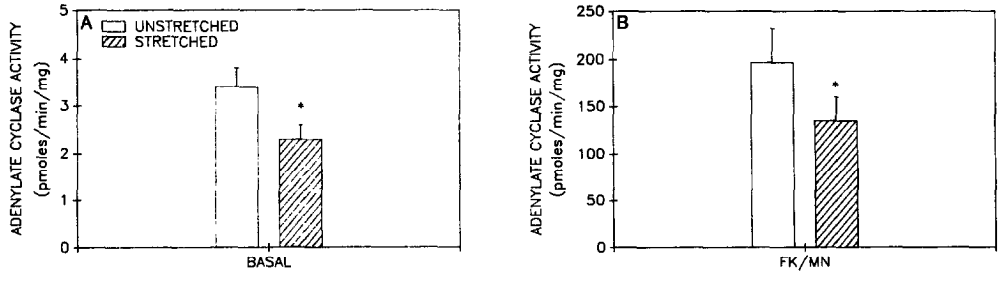
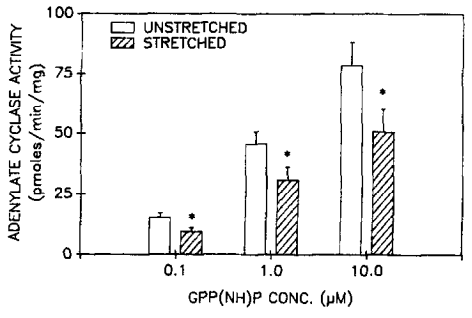
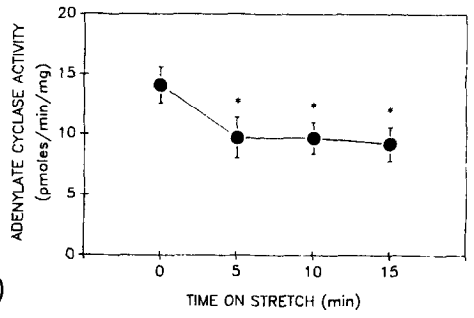


Figure 2. Effect of 30 minute cyclic stretch on (A) basal (BASAL) and (B) maximally stimulated (100 uM forskolin with 5 mM $MnCl_2$ (FK/MN) adenylate cyclase in cultured coronary artery smooth muscle cells. The values represent the mean ± SEM from 8 (BASAL) and 10 (FK/MN) experiments. * = p<0.05 (as compared to unstretched control by paired t-test).



3



4

Figure 3. Effect of 30 minute cyclic stretch on Gpp(NH)p stimulated adenylate cyclase activity in coronary artery smooth muscle cells. The values represent the mean ± SEM; N=7. * = $p<0.05$ (as compared to unstretched control by paired t-test).

Figure 4. Effect of time on stretch on Gpp(NH)p (0.1 uM) stimulated adenylate cyclase activity in cultured coronary vascular smooth muscle cells. The values represent the mean ± SEM; N=7. *=$p<0.05$ (as compared to unstretched control by paired t-test).

rates of ribosome formation presumably by stimulating adenylate cyclase activity (10). In skeletal muscle stimulated to contract for 21 days, adenylate cyclase activity was elevated by changes at the level of the G protein or the catalytic site of the enzyme (11). Our observation of reduced adenylate cyclase activity in response to stretch is opposite to that found in previous studies (9-11). Differences likely reflect the use of different stretch generating models as well as species and tissue specificity.

The precise biochemical mechanism involved in mediating pressure related changes in adenylate cyclase activity observed in the present study cannot be stated with certainty. Mechanosensitive ion channels are present in several cell types including Xenopus oocytes (12), endothelial cells (13) and most notably in smooth muscle cells (14). Further, it is known that stretch may alter physiological and biochemical parameters of cellular function including resting membrane potential (15), Ca^{++} influx via distinct voltage-independent entry channels (16), the conducting state of ion channels in the plasma membrane (14) and myosin light chain phosphorylation at least transiently (17). Recent studies by Yatani et al. (18) and Kim et al. (19) also have demonstrated that guanine nucleotide binding proteins may play a role in activation of K^+ and Ca^{++} channels. Accordingly, stretch could lead to sensitization of adenylate cyclase in coronary vascular smooth muscle by inducing a conformational change in one or more plasma membrane ion channels, in turn influencing transmembrane ion flux and ultimately the activity of the guanine nucleotide binding protein. Additional studies are required to test this hypothesis.

In conclusion, data obtained in this investigation demonstrate for the first time that adenylate cyclase activity in coronary artery smooth muscle cells is sensitive to the mechanical force of cyclic stretch. Responsiveness of the enzyme's activity to mechanical deformation of the cell membrane demonstrates a potential biochemical transduction pathway which may be involved in the myogenic response of vascular smooth muscle to changes in perfusion pressure.

ACKNOWLEDGMENTS: Supported in part by grants from the USPHS NIH-NHLBI [HL 29951] and from the American Heart Association, Rhode Island Affiliate. Dr. Gewirtz is an Established Investigator of the American Heart Association. The authors acknowledge the secretarial assistance of Christine Abatiello and the expert technical assistance of Linda Pietras, Cheryl Cranshaw and Ray Boynton.

REFERENCES

1. Gewirtz, H., Brautigan, D.L., Olsson, R.A., Brown, P., and Most, A.S. (1983) Circ. Res. 53,42-51.

2. Kramer, G.L., and Hardman, J.G. (1980) In Handbook of Physiology (D.F. Bohr, A.P. Somylo, and H.V. Sparks. Eds.) Section 2, Vol II, pp. 179-199. American Physiological Society, Bethesda, MD

3. Gunther, S.R., Alexander, R.W., Atkinson, W.J., and Gimbrone, M.A. (1982) J. Cell Biol. 92,289-298.

4. Mills, I., and Gewirtz, H. (1990) Biochem. Biophys. Res. Comm. 168,1297-1302.

5. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193,265-275.

6. Gilbert, J.A., Banes, A.J., Link, GV.W., and Jones, G.J. (1989) In ANSYS Conference Proceedngs (D.E. Dietrich, Ed), Swanson Analysis System Company, Houston, PA.

7. Mills, I., Garcia-Sainz, A.J., and Fain, J.N. (1986) Biochim. Biophys. Acta 876,619-630.

8. Salomon, Y. (1979) Adv. Cyclic Nucleotide Res. 10,35-55.

9. Somjen, D., Binderman, I., Berger, E., and Harell, A. (1980) Biochim. Biophys. Acta 627,91-100.

10. Watson, P.A., Haneda, T., and Morgan, H.E.(1989) Amer. J. Physiol. 256,C1257-1261.

11. Kraus, W.E., Liggett, S.B., and Williams, R.S. (1989) Clin. Res. 37,272A.

12. Yang, X.C., and Sachs, F.(1989) Science 243,1068-1071.

13. Lansman, J.B., Hallam, T.J., and Rink, T.J. (1987) Nature 325,811-813.

14. Kirber, M.T., Walsh, J.V., and Singer, J.J. (1988) Pflugers Arch. 412,339-345.

15. Harder, D.R. (1984) Circ. Res. 55,197-202.

16. Laher, I., van Breeman, C., and Bevan, J.A. (1988) Circ. Res. 63,669-672.

17. Barany, K., Ledvora, R.F., Mougious, V., and Barany, M. (1985) J. Biol. Chem. 260,7126-7130

18. Yatani, A., Codina, J., Imoto, Y., Reeves, J.P., Birnbaumer, L., and Brown AM (1987) Science 238,1288-1292.

19. Kim, D., Lewis, D.L., Graziadei, L., Neer, E.J., Bar-Sagi, D., and Clapham, D.E. (1989) Nature 337,557-560.