Journal of Academia and Industrial Research (JAIR)
Volume 2, Issue 7 December 2013

ISSN: 2278-5213

RESEARCH ARTICLE

Actomyosin Activity Correlated with Fenoterol Administration

Pooja Sharma* and Sushma Sharma
Dept. of Bio-Sciences, Summer Hill, Shimla-171005, Himachal Pradesh, India
Sharma_poo.magic@rediffmail.com*; +91 9418480688

Abstract

This study investigated actomyosin activity correlated with fenoterol administration in Adult Swiss albino male mice of Balb-C strains. Mice were randomly assigned into two independent groups: One group containing normal mice served as control and the other group as treated groups. Animals of second group were given daily oral administration of fenoterol (1.5 mg/kg body wt) for 28 d. After the experimental period, the specific activity of actomyosin ATPase in the duodenum part of small intestine in the control mice was found to be 47.92 ± 0.96. The specific activity of actomyosin decreased to 16.73 ± 1.82 after fenoterol treatment at 7 d stage. Actomyosin ATPase was found to be 29.69 ± 2.53 in normal jejunum. The activity was decreased from normal value and reached to 6.91 ± 1.72 after 14 d of drug treatment. Ileum is the last part of small intestine where the actomyosin ATPase activity was found to be 54.60 ± 3.98. After fenoterol treatment for 7 d stage, abrupt decrease in the specific activity was observed (1.96 ± 0.02). Actomyosin ATPase activity was decreased at somewhat higher concentrations, after fenoterol treatment from 7 to 28 d of investigation. The magnitude of decrease in ATPase activity increased with increasing daily oral administration of the drug. A decrease in actomyosin ATPase activity was due to the change in myosin isoforms occurring with chronic dilatation and hypertrophy as a result of fenoterol treatment.

Keywords: Actomyosin, fenoterol, actomyosin ATPase, jejunum, ileum, chronic dilation, hypertrophy.

Introduction

It is generally accepted today that contraction of all muscles is brought about by the interaction of actin, myosin and ATP, regardless of the type of muscle or the difference in its physiological behavior. Mammalian smooth muscle cells are contractile cells embedded in the walls of a diverse set of organs including blood vessels, the airways, the gastrointestinal system and the urinogenital system. Smooth muscles from these functionally distinct organs contract in response to a broad array of extracellular messengers including sympathetic and parasympathetic neurotransmitters, autacoids, and hormones. The pattern of contraction varies depending on the source of muscle and the stimulant. There are two general patterns of contraction commonly described as phasic and tonic, which correspond to transient and stable contractions, respectively. The primary intracellular signal for producing contraction is ionic calcium (Ca^{2+}), which activates the Ca^{2+}-calmodulin-dependent enzyme myosin light-chain kinase (MLCK). Activated MLCK catalyzes phosphorylation of the 20 kDa myosin light chains, which increases actin-activated ATPase activity of smooth muscle myosin II. Myosin-associated phosphatases reverse the phosphorylation reaction causing relaxation. It is thought that both the kinase and phosphatase reactions are regulated by enzymes coupled to Ca^{2+}-dependent as well as Ca^{2+}-independent agonist-activated signaling pathways.

There is also indirect evidence that smooth muscle actin associates with several proteins that might regulate myosin II motor function and actin filament structure. The actin-binding proteins caldesmon and calponin are phosphoproteins that inhibit actomyosin ATPase *in vitro*. Both have been hypothesized to be phosphorylated *in vivo* to relieve the inhibition of actomyosin ATPase and to promote contraction or regulate cell shortening (William *et al.,* 2004). One of the main challenges to understand regulation of smooth muscle contraction is the composition and structure of the contractile machinery which is not fully understood. Age-related changes in actomyosin ATPase activity were observed by Prochniewiez *et al*. (2007). Age-related decline of contractility in permeabilized muscle indicates age-related changes in the interaction between muscle actin and/or myosin which make 18-22% (actin) and 43-50% (myosin) of its total protein content (Yates *et al*., 1983). However, since the interaction between actin and myosin in the muscle filament lattice also depends on other structural and regulatory proteins. Against these backdrops, this study investigated actomyosin activity correlated with fenoterol administration in Adult Swiss albino male mice of Balb-C strains.

Materials and methods

Experimental animals: Adult Swiss albino male mice of Balb-C strain weighing 25-30 g were procured from Central Research Institute (CRI), Kasauli, HP, India.

©Youth Education and Research Trust (YERT)

Table 1. Actomyosin ATPase activity in the duodenum, jejunum and ileum of the experimental groups after the treatment periods.

Tissues		Treatment periods			
		7 d	14 d	21 d	28 d
Duodenum	Normal	47.92 ± 0.96	47.96 ± 1.64	47.98 ± 1.21	47.95 ± 0.98
	Treated	16.73 ± 1.82*	10.62 ± 1.14	4.38 ± 0.12	2.70 ± 0.66*
Jejunum	Normal	29.69 ± 2.53	29.69 ± 2.68	29.68 ± 2.42	29.65 ± 2.19
	Treated	6.91 ± 1.72	5.59 ± 0.92*	4.46 ± 0.18*	2.31 ± 0.59*
Ileum	Normal	54.60 ± 3.98	54.62 ± 3.59	54.63 ± 2.89	54.60 ± 2.92
	Treated	1.96 ± 0.02*	1.35 ± 0.02	0.55 ± 0.01*	2.36 ± 0.62*

Values are mean ± SEM; n = 6 (P*<0.05).

They were housed in polypropylene cages under controlled conditions of temperature and light (24 ± 2°C; 16 h d light) and fed upon Hindustan lever pellet diet and water *ad libitum*. All experimental procedures were conducted after the approval of Institutional Animal ethics committee, Himachal Pradesh University (IAEC/BIO/ 4-2006), Shimla.

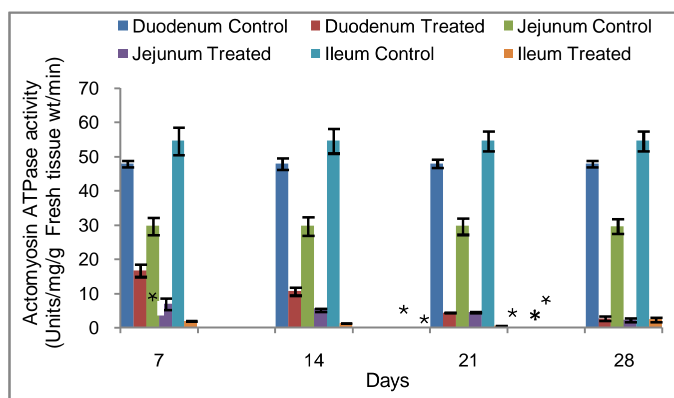
Experimental groups and design: Mice were randomly assigned into two independent groups: One group containing normal mice served as control and the other group as treated groups. Animals of second group were given daily oral administration of fenoterol (1.5 mg/kg body wt) for 28 d. Animals were sacrificed at 7, 14, 21 and 28 d by cervical dislocation. Small intestine (duodenum, jejunum and ileum) were immediately excised. At least 4-6 animals from each group were sacrificed at each stage.

Actomyosin ATPase activity (E.C.3.6.4.1): It was done by the method of Taussky and Shorr (1953). ATPase activity was determined in an incubation medium which comprised of KCl (50 mM), Tris (pH-7) 20 mM, $CaCl_2$ (1 mM), $MgCl_2$ (2 mM), actomyosin (0.2-0.3 mg/mL) and 1 mM ATP. The final volume of the reaction medium was made to 3 mL. ATP was added as substrate to initiate the reaction. The reaction was terminated after 1 min after addition of 15% TCA. The reaction mixture was centrifuged for 10 min at 4000 rpm. To 2 mL of reaction mixture, 3 mL of colouring reagent (2.5% $FeSo_4.H_2O$ containing 5 mL of 10% ammonium molybdate prepared in 10 N H_2SO_4 was added and kept at 37°C for 30 min. The colour absorbance was read at 660 nm. Myosin ATPase activity of respective tissue was calculated directly from the standard curve in terms of μM pi/mg of myosin/min. Potassium phosphate was employed as reference while constructing the calibration curve.

Results

The specific activity of actomyosin ATPase in the duodenum part of small intestine in the control mice was found to be 47.92 ± 0.96. The specific activity of actomyosin decreased to 16.73 ± 1.82 after fenoterol treatment at 7 d stage. Decrease (10.62 ± 1.14) in the specific activity of actomyosin was further observed at 14 d stage. The specific activity was 4.38 ± 0.12 after 21 d of fenoterol administration.

Fig. 1. Specific activity of actomyosin ATPase (Units/mg/g of fresh tissue wt/min) in duodenum, jejunum and ileum of normal and drug treated mice during 7-28 d period.



Values are mean ± SEM; n = 6 (P*<0.05).

Further, decrease in the specific activity (2.70 ± 0.66) was observed at 28 d stage after drug treatment whereas, the control activity was 47.95 ± 0.98 (Table 1, Fig. 1). Actomyosin ATPase was found to be 29.69 ± 2.53 in normal jejunum. The activity was decreased from normal value and reached to 6.91 ± 1.72 after 14 d of drug treatment. The specific activity of actomyosin ATPase further decreased to 5.59 ± 0.92. ATPase activity after 21 d of fenoterol treatment was found to be 4.46 ± 0.18. Great decline (2.31 ± 0.59) in activity was observed at 28 d stage of fenoterol administration (Table 1, Fig. 1). Ileum is the last part of the small intestine, where the actomyosin ATPase activity was found to be 54.60 ± 3.98. After fenoterol treatment for 7 d stage, abrupt decrease in the specific activity was observed (1.96 ± 0.02). After 14 d of drug treatment, the specific activity was declined to 1.35 ± 0.02. At 21 d stage, the specific activity was 0.55 ± 0.01 and at 28 d stage it was slightly increased to 2.36 ± 0.62 mg/g. The values are statistically significant at all the stages in treated mice (P*<0.05) except 14 d stage (Table 1, Fig. 1).

Discussion

In recent years, much interest has been focused on the force generating apparatus responsible for the motility of intestinal epithelial brush border. This highly ordered structure proved to be an ideal model for studying the structural basis of non-muscle cell motility.

Epithelia of rat intestine and of kidney tubules were shown to perform fast microvillar movements (Thuneberg *et al*., 1969; Sandstrom, 1971). Studies on isolated apical segments of intestinal epithelium demonstrated rapid microvillar retraction or contraction of the whole apical segments in response to ATP, Ca^{2+} and Mg^{2+} (Mooseker, 1976; Rodewald and Karnovsky, 1976). Biochemical analysis indicated the presence of myosin, tropomyosin, actin and other associated proteins in the apical cytoplasm (Mooseker, 1976; Mooseker *et al*., 1978; Bretscher *et al*., 1978). These biochemical findings have been confirmed by the immunofluorescent localization of myosin, tropomyosin, and actin in the apical cytoplasm of mouse, rat and chicken intestinal epithelial cells (Bretscher *et al*., 1978; Drenckhahn and Groschel-Stewart, 1980). Actomyosin ATPase activity is decreased at somewhat higher concentrations, after fenoterol treatment from 7 to 28 d of investigation. The magnitude of decrease in ATPase activity increased with increasing daily oral administration of the drug. A decrease in actomyosin ATPase activity was due to the change in myosin isoforms occurs with chronic dilatation and hypertrophy as a result of fenoterol treatment. At the end of 28 d stage, the percentage decrease in the actomyosin activity in duodenum was found to be 94.4%. The percentage decrease in the actomyosin ATPase activity after fenoterol treatment was found to be 92.3% at 28 d stage in jejunum. The percentage decrease was calculated to be 95.68% in ileum at 28 d stage. At 7, 14 and 21 d after drug treatment, the percentage decrease in duodenum was found to be 34.9%, 77.86% and 90.88%. Decrease was found to be 76.8%, 81.2% and 85% in jejunum after drug treatment at 7, 14 and 21 d. While in ileum, the decrease was found to be 96.4%, 97.53% and 99%. This work was in agreement with the work of Gordon *et al*. (2003) where, actomyosin content was decreased by 8.6% and 21% after isoproterenol treatment to the heart of mice**.** ATPase activity from control group had a higher value as compared to treated group. Findings were similar with the work of Patiyal and Sharma (2007) who showed 20% decrease of ATPase activity in the heart after 30 d of clenbuterol treatment. ATPase activity reflects the contractile velocity of a given muscle (Lauer *et al.,* 1989).

Conclusion

Present study demonstrated for the first time that oral administration of fenoterol can enhance muscle apoptosis and hypertrophy. Although the improvements in regenerating fiber size and muscle function associated with a single dose of fenoterol were emphasized. The beta-adrenoceptor agonists have been used to relieve bronchoconstriction. Beta-agonists are based on adrenaline and early forms, such as isoprenaline, lacked bronchial selectivity and had unpleasant side effects. Modern beta-agonists are more selective for the β_2-adrenoceptors located in bronchial smooth muscle and have less cardiotoxicity. However, oral fenoterol administration did have transient effects on some cardiovascular parameters and must be minimized before this form of treatment could be advocated for clinical application. To conclude, advancing our knowledge of control systems of gut motility does not require the creation of simplified models, but it requires increased efforts to understand all the different components separately and in combination. Advanced imaging techniques, advanced motor assessments *in vivo*, advanced molecular techniques, detailed electrophysiology employed *in vivo, in vitro* and *in situ* and the creation of all encompassing models will create the enthusiasm and creativity needed to solve the fascinating control mechanisms of gastrointestinal motor activity and provide solutions for the dramatic presence of motility disorders. Finally, we emphasize the importance of fenoterol in motility studies. We also stress the importance of collaboration and a multidisciplinary approach for future understanding of the mechanisms of the small intestine in health and diseases.

References

1. Bretscher, A. and Weber, K. 1978. Localization of actin and microfilament associated proteins in the microvilli and terminal web of the intestinal brush border by immunofluorescence microscopy. *J. Cell Biol.* 79: 839-845.
2. Drenckhahn, D. and Groschel-Stewart, U. 1980. Immunocytochemical localization of myosin in the brush border region of intestinal epithelium. *Cell Tissue Res.* 205: 163-166.
3. Gordon, L.M., Inchiosa, J.R. and David, L. 2003. Isoproterenol induced cardiomegaly: Assessment of myocardial protein content, actomyosin ATPase and heart rate. *J. Cell Cardiol.* 4: 543-557.
4. Lauer, B., Vanthiem, N. and Swynghedauw, B. 1989. ATPase activity of the cross-linked complex between cardiac myosin sub-fragment 1 and actin in several models of chronic overloading. A new approach to the biochemistry of contractility. *Circ. Res.* 64: 1106 -1115.
5. Mooseker, M.S. 1976. Brush border motility microvillar contraction in Triton-treated brush borders isolated from intestinal epithelium. *J. Cell Biol.* 71: 417-433.
6. Mooseker, M.S., Pollard, T.D. and Fujiwara, K. 1978. Characterization and localization of myosin in the brush border of intestinal epithelial cells. *J. Cell Biol.* 79: 444-453.
7. Patiyal, S.N. and Sharma, S. 2007. Chronic oral administration of beta–adrenoceptor agonist clenbuterol affects myosin heavy chain (MHC) expression in adult mouse heart. *Physiol. Res.* 56: 275-283.
8. Prochniewiez, E., La Dora, A., Thompson, B. and David, D. 2007. Age related decline in actomyosin structure and function. *Exp. Gerontol.* 9: 684-690.
9. Rodewald, R.N. and Karnovsky, M.J. 1976. Contraction of isolated brush borders from the intestinal epithelium. *J. Cell Biol.* 70: 541-554.
10. Sandstrom, B. 1971. A contribution to the concept of brush border function observation on intestinal epithelium in tissue culture. *Cytobiolope.* 3: 293-297.
11. Taussky, H.H. and Shore, E. 1953. A micro-choloromitric method for the determination of inorganic phosphorus. *J. Biol. Chem.* 202: 675-685.
12. Thuneberg, L. and Rostgard, J. 1969. Motility of microvilli. A film demonstration. *Ultrastruct. Res.* 29: 578.
13. William, T., Gerthoffer, L. and Janice, K. 2004. Regulation of smooth muscle contraction. *Adv. Org. Biol.* 8: 49-80.
14. Yates, L.D. and Greaser, M.L. 1983. Quantitative determination of myosin and actin in rabbit skeletal muscle. *J. Mol. Biol.* 168: 123-141.