Connective Tissue Generates Mechanical Tension during Contraction of the Vascular Wall

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It is shown that the contractions of isolated aorta strips, induced by phorbol ester (phorbol-12-myristate-13-acetate), a synthetic diacylglycerol mimetic, are not attended by changes in the rigidity of the strips.

Key Words: *connective tissue; aorta wall; phorbol esters; mechanical tension; rigidity*

Great attention has been paid to the role of Ca^{2+}-phospholipid-dependent kinase (proteinkinase C) in the regulation of smooth muscle cell contraction [10,11].

The contractile responses of the smooth muscle tissue caused by activators of proteinkinase C exhibit characteristics which are difficult to explain on the basis of current knowledge about the mechanism of actin-myosin interaction. The most notable of these characteristics are a constant concentration of intracellular Ca^{2+} and a constant level of phosphorylation of the light chains of myosin during the development of contraction, as well as the fact that contraction does not depend on the degree of integrity of the endothelium [3-5]. On the other hand, similar regularities have been observed for thermomechanical responses of the aorta wall [12], which, as we have established, are due mainly to the connective tissue matrix [15]. We have previously suggested that the contractile activity of the smooth muscle tissue is the sum of two active synchronized motor responses: the response of the smooth muscle cells, resulting from the actin-myosin interaction, and the response of the connective tissue matrix, in which the contractile functions are fulfilled by the three-dimensional fibrillar network formed by types I and III collagen [14].

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In view of the foregoing we suggested that, as in the case of thermomechanical responses of the aorta wall, mechanical responses of the connective tissue matrix underlie the smooth muscle tissue contractions induced by proteinkinase C activators.

MATERIALS AND METHODS

The experiments were carried out on isolated preparations of guinea pig aorta. Rectangular strips (1×6 mm) were excised parallel to the large axis of the vessel and mounted in the experimental chamber, one end being hooked up to a Mioton-TsA-012-MT force transducer (Academic Center, Ekaterinburg) and the second to a Mioton-TsA-012-3D mechanical deformation transducer (the same manufacturer). The strip was stretched from the initial length L_{ini} to the length L_o, which was optimal for generating the maximum force. The length L_{ini} was determined after Pawlowski et al. [12], while the length L_o was the same as that chosen by Resnick et al. [13] and constituted 1.4 L_{ini}. Before measurements the preparations, stretched to L_o, were incubated for 60 min in normal Krebs solution of the following composition (mM): $NaCl_2$ 120.4, KCl 5.9, $NaHCO_3$ 15.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 11, and $CaCl_2$ 2.5.

Calcium-free (relaxing) solution was prepared by replacing 2.5 mM Ca^{2+} in the Krebs solution

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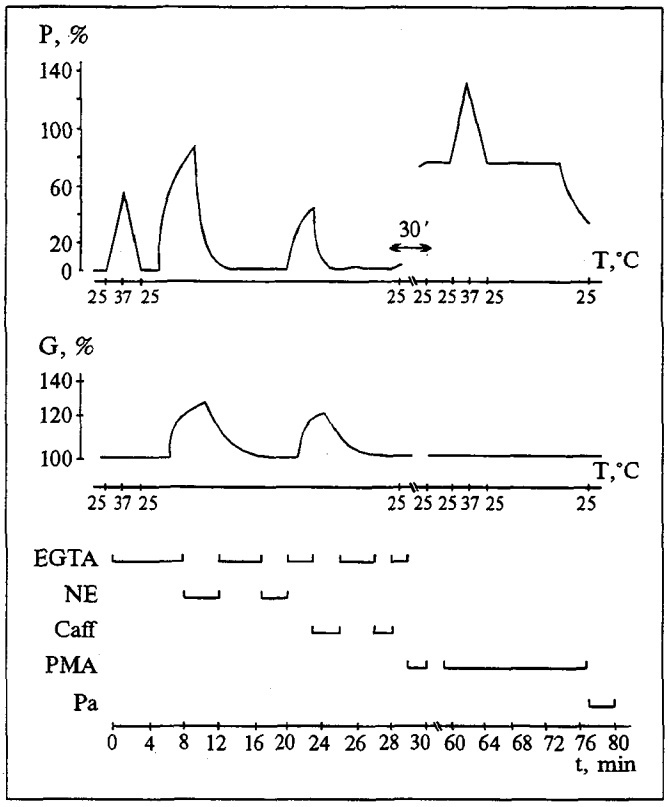

Fig. 1. Typical recording for one of seven experiments during studies of the effect of PMA in isolated aorta strips. *t*: temperature, °C; *P*: mechanical tension, % of maximum (maximum tension of strips attained in the presence of hyperpotassium solution and 10^{-5} M norepinephrine in combination); *G*: rigidity (rigidity of preparations in the absence of stimulation taken as 100%). *EGTA*: CA–free solution; NE: EGTA with norepinephrine (10^{-5} M); Caff: EGTA with caffeine (10^{-5} M); PMA: EGTA with phorbol–12–myristate–13–acetate (5×10^{-6} M); Pa: EGTA with papaverin (10^{-4} M).

with 1 mM ethyleneglycol-bis(β-aminoethylether)-N,N'-tetraacetic acid (EGTA), and hyperpotassium solution by a 75% equimolar replacement of NaCl in the Krebs solution with KCl.

EGTA, phorbol-12-myristate-13-acetate (PMA) (a synthetic diacylglycerol mimetic), norepinephrine, caffeine, and papaverin were purchased from Sigma Chemical Co.

The strips were tested under isometric conditions at L_0. The experimental chamber was thermostatically controlled with an automatic device allowing the temperature in the chamber to be changed from 0 to +45°C.

The rigidity of the preparations was studied by exposing them to sinusoidal oscillations (frequency 30 Hz and amplitude 0.5% of L_0) [13].

RESULTS

A typical recording for one of the seven experiments is presented in Fig. 1.

After a 60-min incubation in normal Krebs solution, the latter was replaced with a calcium-free solution, in which the preparation was incubated for 10 min before thermal exposure. The mechanical response of the strip to changes of temperature over the range 25-37°C was not attended by an increase of rigidity, whereas during contractions caused by norepinephrine and caffeine, marked changes of the rigidity of the preparation were observed.

Each of the two agonists failed to cause more than one contraction of the preparations, which can evidently be attributed to depletion of the intracellular sources of Ca^{2+} (Fig. 1). However, the development of the contractile response in the presence of PMA was the same as that in the presence of Ca^{2+}. The most important fact is that the rigidity of the strips during PMA-induced contractions remained unchanged. The same was observed during thermomechanical responses of these preparations on the plateau of the PMA-induced contraction. In the case of PMA-induced contractions, papaverin caused a marked, though incomplete, relaxation; nevertheless, the rigidity of the strip remained constant.

A constant rigidity, along with the development of force, is, as we have established, a characteristic feature of the mechanical responses developed by the connective tissue matrix [15]. Since the thermomechanical responses of the aorta wall are mainly due to the connective tissue, this readily explains the unchanged rigidity in the case of thermoinduced mechanical responses of the aorta strips.

The ability of the aorta strip preparations to generate mechanical tension without changing their rigidity when Ca^{2+} is absent in the external medium and the intracellular sources of Ca^{2+} are depleted corroborates, in our opinion, the foregoing assumption that the phorbol ester-induced contraction in the aorta wall is also due to the connective tissue matrix. This experimental finding does not seem to find an explanation in the classical hypothesis of actin-myosin interaction, since the increase of rigidity during contraction of the muscle tissue is crucial evidence of its actinomyosin nature [9].

It cannot be assumed that during these mechanical responses slight changes in the rigidity of preparations which result from actin-myosin interaction are entirely "masked" by the high passive rigidity of the strips caused by stretching [12]. Changes of this index in the case of norepinephrine- and caffeine-induced contractions provide persuasive evidence that such a theory is erroneous (Fig. 1).

Since PMA, as we established, does not directly act upon the connective tissue, it can be assumed that there is a PMA-activated system which controls the mechanical state of the connective tissue matrix (possibly via certain transmitters) in the smooth muscle cells. An important role in this system seems likely to be played by proteinkinase C, because its activation goes along with the development of mechanical tension, generated, we believe, by connective tissue structures.

Since the connective tissue matrix actively contributes to the contractile process of the smooth muscle tissue, this underlies the need to supplement the well-known Hill model [7], in its application to smooth muscles, with a second contractile element. It cannot be ruled out that a third force-generating active element (reflecting the possible contractile responses of the intermediate vimentin-desmin filaments during the contraction of smooth muscle cells) is also to be taken into account.

It seems very likely that during contraction of the muscle tissue, the extracellular connective tissue, as well as the intracellular connective fibrillar structures (for example, titin), also actively participate in the generation of mechanical tension in some types of skeletal or cardiac muscle. This appears even more likely if we remember that collagen is present in the intercellular space of these muscles [1,6] and that there are marked physicochemical shifts in the cytoplasm of the muscle cells, usually going along with contraction [2,5]. These factors may underpin the conformational changes occurring in individual molecules and the mechanical reactions taking place in the network of fibrillar structures as a whole.

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