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TPA- AND AGONIST-INDUCED FORCE DEVELOPMENT IN MYOMETRIUM FROM PREGNANT AND NON-PREGNANT RATS

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Summary In myometrium from pregnant rats, 100 nM-TPA elevated resting tension and initially slightly enhanced the contraction induced by 138 mM-KCl. After 20 min this force development significantly declined. In saponin-treated skinned myometrial cells from pregnant rats, 100 nM-TPA enhanced the contraction induced by 0.3 μM-Ca^{2+}, but reduced that induced by 1 μM-Ca^{2+}. These findings suggest that the excitatory and inhibitory actions of TPA on the myometrium are probably due to its action on the contractile proteins. In myometrium from non-pregnant rats, TPA affected neither the resting tension, nor the amplitude of the evoked contractions, nor the Ca^{2+}-induced contractions in skinned myometrium. While TPA only affected tension development in pregnant rats, both 1 mM-carbachol and 90 nM-oxytocin induced a tonic contraction in Ca-free solution independently of the hormonal status of the rats. The latter finding makes it unlikely that activation of protein kinase C is involved in the agonist-induced tonic force development in Ca-free solution.

In smooth muscles, as in many other tissues, a guanine nucleotide-binding protein is activated upon binding of agonists to their receptors (1). The activated guanine nucleotide-binding proteins enhances also in smooth muscle the activity of a polyphosphoinositide phosphodiesterase (2). The subsequent hydrolysis of inositol-containing phospholipids generates diacylglycerol and inositol phosphates. It remains as yet unknown whether the activation of protein kinase C by diacylglycerol modifies the tension of smooth muscle.

The analysis of agonist-induced contractions in Ca-free medium provides information on contractions, which cannot depend on Ca^{2+}-influx through Ca^{2+}-channels. The force development induced in some smooth muscles by agonists after a prolonged incubation in Ca-free solutions has been explained by a Ca-independent mechanism (3-6), as well as by a Ca-dependent one (7,8).

The aim of the present work was to investigate further the action of 12-o-tetradecanoyl phorbol-13-acetate (TPA), an activator of protein kinase C, in myometrium and to find out whether it is involved in the generation of the tension response in Ca-free solutions. Because, at least in mouse mammary gland, protein kinase C activity is stimulated by oestrogens (9), we have

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furthermore investigated whether the effect of TPA on myometrium is affected by the occurrence of pregnancy in the animals.

Materials and Methods

Animals. The longitudinal smooth-muscle layer was prepared from uteri of pregnant and non-pregnant rats as described previously (10).
Solutions. The composition of the modified Krebs-solution was as follows: 135.5 mM-NaCl, 5.9 mM-KCl, 1.5 mM-$CaCl_2$, 1.2 mM-$MgCl_2$, 11.5 mM-glucose and 11.6 mM-Hepes (pH 7.3). The solution was bubbled with O_2. Changes of $[K^+]$ were compensated by equivalent modifications of $[Na^+]$ in order to maintain constant molarity. Ca-free solutions always contained 2 mM-EGTA. In skinned muscles, the following relaxing solution was used: 114 mM-K-methanesulphonate (KMs), 20 mM-Tris maleate, 5.1 mM-$Mg(Ms)_2$, 5.2 mM-ATP and 4 mM-EGTA. Various Ca^{2+}-concentrations were prepared by adding appropriate amounts of $CaMs_2$ to 4 mM-EGTA.
Recording of mechanical activity. Mechanical activity of intact and skinned smooth muscle was measured by attaching the muscle strip (0.3 - 0.5 mm in length, 0.05 - 0.08 mm in width and 0.03 - 0.05 mm in thickness) to a strain-gauge (U-gauge, Minebea Co. Ltd., Tokyo, Japan) in a chamber with 0.9 ml capacity. The solution was changed by perfusing the chamber from one end with a syringe and sucking simultaneously from the other end. To rule out artifacts due to the sudden changes of the solutions, the recovery of the position of the recording pen to the original level was checked, and the position was eventually adjusted. Skinned muscle fibres were prepared by treating the strips with 30 μg/ml of saponin in relaxing solution for 20 min (11). The Ca^{2+}-induced contractions were measured at various concentrations of free Ca^{2+}, using EGTA-buffer solutions. TPA was applied during the Ca^{2+}-induced contractions after the tension development reached a constant value.
Drugs. Chemicals used were oxytocin and 12-o-tetradecanoylphorbol-13-acetate (TPA) (Sigma Chem. Co., St. Louis, U.S.A.), saponin (ICN Pharmac. Inc., Cleveland, OH, U.S.A.), carbachol (BDH Chemicals Ltd. Poole, England) and ATP (Boehringer, Mannheim, Germany).

Results

Effect of TPA on intact myometrial strips.

Smooth-muscle strips from myometrium of non-pregnant and pregnant rats were incubated in a modified Krebs-solution, in which the K^+-concentration was increased for 3 min from 5.9 mM to 138 mM. Thereupon the fibres were again exposed for 7 min to a solution containing 5.9 mM-K^+. This procedure was repeated many times. The effects of 100 nM-TPA on the resting tension and on the amplitude of contractions evoked by 138 mM-K^+, are represented in Fig 1. TPA does not affect the resting tension in myometrium from non-pregnant rats, nor the amplitude of the evoked contractions. In contrast, 100 nM-TPA gradually elevates resting tone in myometrium from pregnant rats. The contractions induced by KCl were initially slightly enhanced, but later on they were appreciably inhibited.

Effect of TPA on contractile proteins in skinned myometrial fibres.

The minimum Ca^{2+}-concentration required to induce contraction in skinned myometrial fibres from pregnant rats was 0.3 μM, and a maximal force was

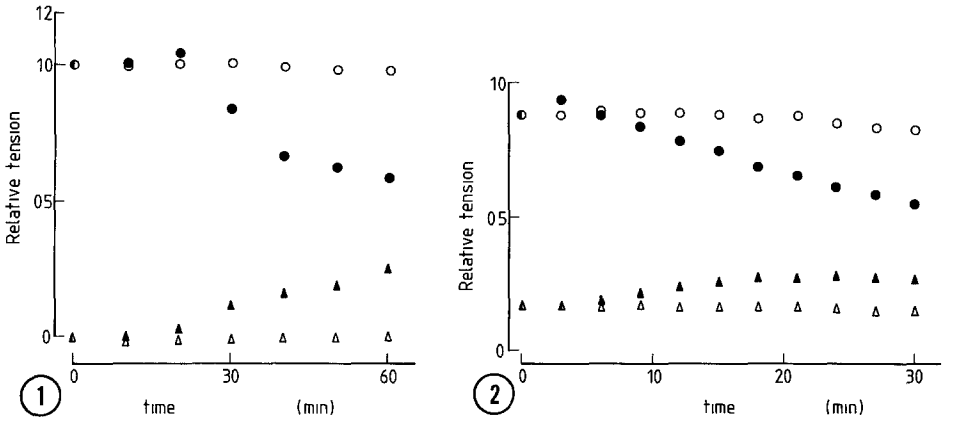

Fig. 1. Effect of 100 nM-TPA on resting tension (△,▲) and on the amplitude of contractions elicited by a solution containing 138 mM-K^+ (○,●) in myometrium from pregnant (▲,●) and non-pregnant (△,○) rats. The maximum amplitude of these contractions is normalized to that induced by 138 mM-K^+ in the absence of TPA, and is given on the ordinate. 100 nM-TPA was applied at time 0, and the time (in min) after adding TPA is represented on the abscissa. The values represent the mean of 5 determinations.

Fig. 2. Effect of 100 nM-TPA on the contractions induced in saponin-skinned myometrium from pregnant rats by 0.3 μM-Ca^{2+}- (▲,△) and 1 μM-Ca^{2+} (●,○). ▲ and ● represent the amplitude of the contractions, as a function of time after adding 100 nM-TPA. TPA was added after the Ca^{2+}-induced contraction had reached a steady-state level. △ and ○ represent the amplitude of the contractions as a function of time in the absence of TPA. The amplitude of the Ca^{2+}-induced contractions is represented on the ordinate relative to the amplitude of the contractiom induced in intact muscle by a solution containing 138 mM-K^+ and 1.5 mM-Ca^{2+} (n=5). The time after addition of TPA (min) is represented on the abscissa.

observed at 10 μM. Fig 2 illustrates the effect of TPA on these Ca^{2+}-induced contractions at 0.3 μM- and at 1 μM-Ca^{2+} in myometrial strips from pregnant rats. 0.3 μM- or 1 μM-Ca^{2+} applied to skinned fibres evoked contractions of almost constant amplitude for over 30 min. Addition of TPA after the contraction had reached a steady state slightly enhanced the contraction induced by 0.3 μM-Ca^{2+}. However, in the presence of 1 μM-Ca^{2+}, the amplitude of the Ca^{2+}-induced contraction was first transiently elevated by TPA and then gradually reduced. In contrast, TPA did not exert an effect on skinned fibres from non-pregnant animals that were contracting by 0.3 μM- or 1 μM-Ca^{2+}.

Tonic force development in myometrial strips from pregnant and non-pregnant rats induced by carbachol and oxytocin in Ca-free solution.

The rate of force development and the duration of the contraction determine whether a contraction in Ca-free solution is considered as phasic or tonic. While phasic contractions in Ca-free medium are characterised by a very rapid rise of tension development, followed by a rapid relaxation, the tonic force development reached its maximum only after 172 ± 10 sec (n=4) and the amplitude remained constant as long as either carbachol or oxytocin were

present. It was observed that both agonists induced a tonic contraction in tissues from pregnant and non-pregnant rats exposed to Ca-free solution.

Discussion

TPA increases resting tension in myometrium from pregnant rats. Such increase by phorbolesters has been reported in many vascular tissues (12-21), in calf trachea (22) and in rabbit iris (23). In contrast, no effect on resting tension could be observed in rat tail artery (24), guinea pig ileum and vas deferens (14). The absence of an effect on resting tension in myometrium from non-pregnant rats in this study has also been reported by Baraban et al. (14). KCl-induced contractions are reported to be increased by TPA in guinea pig trachea and ileum (25) and in rat vas deferens (14), but not in myometrium from non-pregnant rats (14, this study). An increase of the contractions elicited by K-rich solutions and the subsequent decrease of this amplitude which is observed in myometrium from pregnant rats in this study, has already been described in rabbit mesenteric artery (15).

Our observation that TPA exerts similar effects in intact tissues and skinned myometrium suggests that its action in intact cells is mainly due to its effect on the contractile proteins. Recently, evidence has been brought forward that the protein kinase C-induced phosphorylation of myosin light chains may play an inhibitory role in the contraction of vascular smooth muscle (26). However the mechanism of the enhancement of basal tension in intact strips and of the contractions induced by 0.3 μM-Ca^{2+} in skinned fibres is still unknown.

An important conclusion in this work is that TPA only affected tension development in myometrium from pregnant rats and not in that of non-pregnant animals. It could be proposed that pregnancy increases dramatically either the concentration of protein kinase C, or that of its target molecules. Whether this is a consequence of stretching the myometrium, or of the changing hormonal environment has to be clarified. It is interesting to note that oestrogens are reported to stimulate protein kinase C activity in mouse mammary gland (9).

A tonic contraction in smooth muscle exposed to Ca-free medium can be established under the two hormonal conditions, as well with oxytocin as with carbachol. Such a tonic force development of myometrial strips induced by oxytocin (4,27), PGE_1 (28), acetylcholine and $PGF_{2\alpha}$ (7) has already been described but the mechanism responsible for them is as yet unknown. It is unlikely that the protein kinase C pathway is involved in these tonic contractions in Ca-free solutions, because in myometrium from non-pregnant rats, TPA does not affect tension development, while oxytocin and carbachol do induce a tonic contraction in Ca-free solution.

We can conclude that pregnancy makes the myometrium sensitive to TPA. The action of TPA on intact strips can be explained by its effect on the

contractile filaments. Activation of protein kinase C is not involved in the agonist-induced tonic force development in Ca-free solution.

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