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Short communication

Mechanisms involved in UTP-induced contraction in isolated rat aorta

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Abstract

The mechanisms of UTP-induced contractions in the rat aorta strips were studied. These were only partially inhibited in a Ca^{2+}-free medium or by incubation with verapamil or nifedipine. Successive challenges did not decrease the magnitude of the contraction in the absence of external Ca^{2+}. Quin 2(acetoxymethyl) ester (Quin 2AM), 8-(*N,N*-diethylamino)octyl 3,4,5-trimetoxybenzoate (TMB-8), thapsigargin and ryanodine inhibited these contractions. The participation of protein kinase C is also very likely, since downregulation by the phorbol 12,13 dibutyrate (PDB) decreased UTP-induced contraction, and staurosporine and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) antagonized UTP-induced contractions and relaxed UTP-induced tonic contractions. Therefore, different pools of intracellular Ca^{2+} and protein kinase C seem to participate in UTP-induced contraction and in the mechanisms of maintenance in a Ca^{2+}-free medium.

Keywords: UTP contraction; Aorta, rat; Smooth muscle; Vascular; Protein kinase C; Ca^{2+}

1. Introduction

Adding UTP to an isolated rat aorta in an organ bath causes two effects dependent on the experimental conditions. When UTP was added to the organ bath directly, it caused a tonic contraction that is not affected by the presence of the endothelium. An endothelium-dependent relaxation was generated when the tone was raised by noradrenaline (García-Velasco et al., 1995). Pyrimidine nucleotides are found at the extracellular level (Lazarowski and Harden, 1999) and, since they modulate the vascular tone, they are of physiological relevance. In an attempt to characterize the receptor involved in UTP-induced contraction, it was observed that the response is pertussis-toxin-sensitive (López et al., 1998). Therefore, the receptor belongs to the P2Y subfamily of G-protein-coupled receptors (Nicholas et al, 1996). Based on the agonist and antagonist profile, the functional characterization of the suspected receptor (García-Velasco et al., 1995; López et al., 1998) does not match any of the nucleotide receptors identified in rat so far (Fredholm et al., 1997; Bogdanov et al., 1998).

The aim of the present study was to pharmacologically characterize the transduction mechanisms related to UTP-induced contractions in isolated rat aorta. It has been reported that UTP elicited intracellular Ca^{2+} mobilization in rat aorta (Kitajima et al., 1994). Our main concern was to correlate the relationship between Ca^{2+} mobilization and inositol trisphosphate and protein kinase C dependent mechanisms.

2. Methods

Rat aorta strips preparations (Garcia-Velasco et al., 1995) of 2-month-old male Wistar rats were used. They were mounted in 6 ml organ baths containing Krebs solution (with the following composition (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.5; KH_2PO_4, 1.19; $NaHCO_3$, 25; $MgSO_4$, 1.2 and glucose, 11) at 37°C and bubbled continuously with a 95% O_2 and 5% CO_2 mixture. The tissues were allowed to equilibrate for 120 min under 2 g basal tension before experimentation (Garcia-Velasco et al., 1995). Isometric tension was recorded by force-displacement transducers (UF1) on an OmniScribe D-5000 polygraph.

Cumulative concentration–response curves for UTP (3 μM to 10 mM) were carried out in Krebs solution with 2.5

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mM Ca^{2+} or 0 mM plus 0.5 mM EGTA. In addition, contractions to UTP (0.3 mM) in Krebs containing different Ca^{2+} concentrations (2.5 and 0 mM plus 0.5 mM EGTA) were performed. The tissues were allowed to equilibrate in each incubation medium for 30 min. The EGTA was added to the organ bath 2 min before each challenge to UTP (0.3 mM). The effect of verapamil (1 μM) and nifedipine (0.1 μM) on the contractions induced by UTP (0.3 mM) was also assayed.

To evaluate the contribution of intracellular Ca^{2+} to the UTP-induced contraction, the effects of Quin 2(acetoxymethyl) ester (Quin 2AM) (50 μM), 8-(*N*,*N*-diethylamino)octyl 3,4,5-trimetoxybenzoate (TMB-8) (100 μM), dantrolene (100 μM), sodium azide (3 mM), thapsigargin (3 μM) and ryanodine (100 μM) were studied in a Ca^{2+}-free medium plus 0.5 mM EGTA. In addition a calmodulin inhibitor, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) (100 μM), and protein kinase C inhibitors, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) (10 and 100 μM) and staurosporine (0.1 and 1 μM), and an agonist of protein kinase C, the phorbol ester 12,13-dibutyrate (PDB) (0.1 μM) were also studied. In each preparation a single drug was assayed. The different agents were allowed to incubate 30 min before a second UTP challenge, with the exception of Quin 2AM and PDB, 3 and 2 h respectively. The contractile effects of ryanodine (100 μM), thapsigargin (3 μM) and PDB (0.1 μM) were also tested in a Ca^{2+}-free medium plus 0.5 mM EGTA. The desensitization to PDB (0.1 μM) was also analyzed.

Additional experiments were performed to study the relaxant effect of TMB-8 (1 μM to 0.3 mM), W-7 (10 μM to 0.3 mM), H-7 (0.1 to 30 μM) and staurosporine (3 nM to 0.3 μM) on UTP (0.3 mM)-induced tonic contractions in a Ca^{2+}-free medium plus 0.5 mM EGTA. To this end,

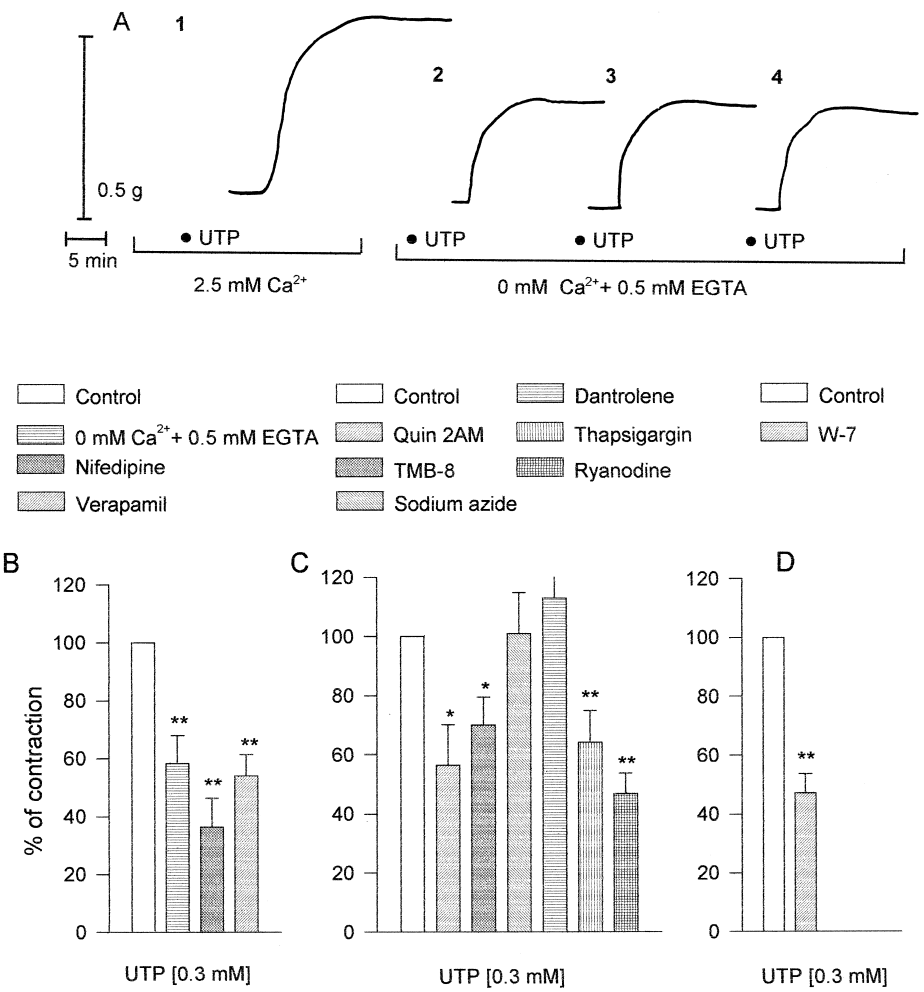

Fig. 1. (A) UTP (0.3 mM)-induced contraction in Krebs solution (2.5 mM of Ca^{2+}) (1) and the successive challenges (30 min interval) to UTP in Ca^{2+} free medium (+0.5 mM EGTA) (2,3,4). Recordings are representative of at least six similar experiments. (B) Percentage of response to UTP (0.3 mM) in: 2.5 mM of Ca^{2+} (Control), 0 mM of Ca^{2+} + 0.5 mM of EGTA, and effects of incubation with nifedipine (0.1 μM) and verapamil (1 μM). (C) Effects of chelators and modulators of intracellular Ca^{2+} on UTP (0.3 mM)-induced contraction in Ca^{2+} free medium (+0.5 mM EGTA). Incubation with Quin 2 AM, (50 μM) for 3 h, TMB 8 (100 μM), dantrolene (100 μM), sodium azide (3 mM), thapsigargin (3 μM) and ryanodine (100 μM), during 30 min on UTP-induced contractions. (D) Effects of incubation (30 min) with W-7 (100 μM). Vertical bars represent average ± S.E.M. from six experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ by means of Student's *t* test.

cumulative concentrations of these agents were added to UTP raised tone.

3. Results

UTP-induced contractions in rat aorta strips was significantly decreased in a Ca^{2+}-free medium containing 0.5 mM EGTA. The concentration–response curves for UTP were shifted to the right and the maximal effect was decreased in comparison to those obtained in 2.5 mM $CaCl_2$ (data not shown). The contractions could be repeatedly elicited inducing challenges every 30 min, for at least 4 h of continuous incubation of the preparation in a Ca^{2+}-free medium (Fig. 1A). These contractions were stable for long periods of time. The magnitude of UTP (0.3 mM)-induced tonic contractions measured at 5, 10, 15, 20, 25 and 30 min, after the maximum effect was reached, were 92.4 ± 1.9, 88.8 ± 3.2, 86.1 ± 4.1, 86.2 ± 6.6, 86.3 ± 5.4 and 86 ± 6.1%, respectively.

The incubation of rat aortic strips with a Ca^{2+}-free medium plus 0.5 mM EGTA with nifedipine (0.1 μM) and verapamil (1 μM) reduced UTP-induced tonic contractions. (Fig. 1B).

The intracellular Ca^{2+} modulators Quin 2AM (50 μM) and TMB-8 (10 μM) reduced the contractile effect induced by UTP in a Ca^{2+}-free medium plus 0.5 mM EGTA (100%). Dantrolene (0.1 mM) and sodium azide (3 mM) did not modify UTP-induced contractions (Fig. 1C). Thapsigargin (3 μM) induced a slow contractile effect in rat aorta strips [199 ± 51% in comparison to the effect induced by UTP 0.3 mM (100%)]. Incubation with thapsigargin (3 μM) inhibits UTP-induced contraction. Ryanodine (100 μM) induced also a contractile response, 60.30 ± 15.2%, and the incubation with it reduced UTP-induced contraction (Fig. 1C). W-7 (0.1 mM) also inhibits these contractions (Fig. 1D).

The protein kinase C inhibitors, H-7 (10 μM and 0.1 mM) and staurosporine (0.1 and 1 μM), inhibited UTP-induced contractions in a concentration-dependent way (Fig. 2A). PDB (0.1 μM) induces contraction in aorta strips in a Ca^{2+}-free medium. This contraction was seven times larger than that induced by UTP (0.3 mM). Incubation for 2 h with PDB 0.1 μM decreased to 62.2 ± 9.2 % a later challenge to PDB (0.1 μM) and UTP-induced contractions (Fig. 2A).

TMB-8 (1 μM to 0.3 mM), W-7 (10 μM to 0.3 mM), staurosporine (3 nM to 0.3 μM) and H-7 (0.1 to 30 μM), induced a concentration-dependent relaxation of UTP-raised tone. The order of potency was: staurosporine > H-7 > TMB-8 = W-7 (Fig. 2B). The IC_{50} obtained was 0.2 nM, 1.7, 33.5 and 59.1 μM, respectively.

4. Discussion

UTP-induced tonic contractions in rat aorta strips are mainly due to the release of intracellular Ca^{2+}. This is suggested by the fact that extracellular Ca^{2+} depletion (from 2.5 to 0 mM plus EGTA) or the incubation of the

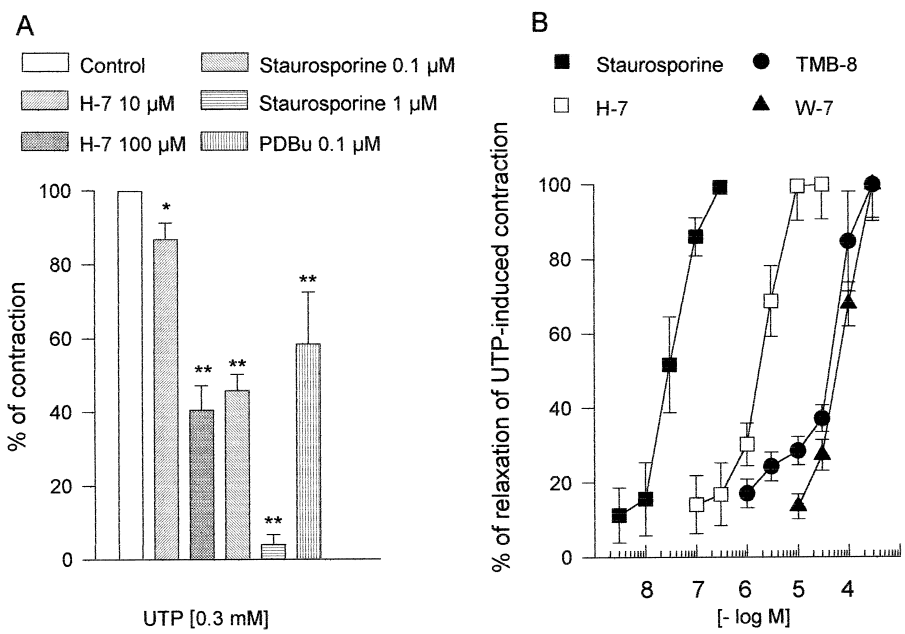

Fig. 2. (A) Effects of protein kinase C inhibitors and PDB desensitization on UTP (0.3 mM)-induced contraction in Ca^{2+} free medium (+0.5 mM EGTA). Incubation with H-7 and staurosporine, during 30 min and 2 h incubation with PDB (0.1 μM). (B) Concentration–response relaxation curves induced by staurosporine (3 nM to 0.3 μM), H-7 (0.1 to 30 μM), TMB-8 (1 to 300 μM) and W-7 (10 to 300 μM) on UTP (0.3 mM)-induced tonic contraction in rat aorta. Values are expressed as a percentage of the maximal relaxation (100% baseline). Vertical bars represent average ± S.E.M. from at least six experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ by means of Student's t test.

preparations with Ca^{2+} entry blockers partially reduced the magnitude of the contractions in a similar way. Similar to rat aorta, in other vascular smooth muscle preparations, UTP also induced maintained contractions in a Ca^{2+}-free medium or in the presence of Ca^{2+} entry blockers (Saïag et al., 1990). Different dependences to external Ca^{2+} [sensitive (Saïag et al., 1990) and insensitive (Sánchez-Fernández et al., 1993)] have been reported to occur in vascular smooth muscles in response to UTP.

UTP-induced contractions are not affected by continuous incubation in Ca^{2+}-free medium, since there is no decrease in the magnitude of the response after repeated challenges to UTP. In addition, as expected, a chelator of intracellular Ca^{2+}, Quin 2AM inhibited the UTP-induced contraction. These findings suggest that UTP, in the absence of external Ca^{2+}, produces contraction via the release of intracellular Ca^{2+}, as reported for other vascular preparations (Saïag et al., 1990). Afterwards, Ca^{2+}-calmodulin system is related to the UTP effect in rat aorta, since it was inhibited by W-7. Furthermore, TMB-8 and W-7 relaxed UTP-induced tonic contraction. The mitochondria do not seem to play a functional role since sodium azide, an inhibitor of the mitochondrial energy-dependent accumulation of Ca^{2+}, did not modify UTP-induced contractions.

Two main mechanisms are related to the release of intracellular Ca^{2+}: via stimulation of inositol trisphosphate receptors and via Ca^{2+}-induced Ca^{2+} release. It has been proposed that inositol trisphosphate is the main mechanism related to the intracellular Ca^{2+} release by UTP in vascular smooth muscle cells (Strobaek et al., 1996). Intracellular Ca^{2+} release seems to play a significant role in UTP-induced contractions in rat aorta, since the response is sensitive to the incubation of the preparations with a nonspecific inhibitor of internal Ca^{2+} release, such as TMB-8, and to the depletion of Ca^{2+} stores with thapsigargin or ryanodine. In addition, ryanodine and thapsigargin elicited contraction in our preparation. These findings suggest that functional Ca^{2+} stores sensitive to ryanodine (Ca^{2+}-induced Ca^{2+} release) and to thapsigargin (inositol trisphosphate-sensitive) are present in the rat aorta and involved in UTP-induced contraction.

Besides inositol trisphosphate, protein kinase C is also involved in the contraction induced by several contraction agonists via the activation of phospholipase C and the production of diacylglycerol. As a result, the activation of protein kinase C might cause an increase in the open channel probability, via the phosphorylation of L-type Ca^{2+} channels, and Ca^{2+} release from the reticulum, via phosphorylation of inositol trisphosphate receptors. In addition, effects on Ca^{2+} pumps, and Na^{+}/Ca^{2+} exchange were also reported. Furthermore, protein kinase C may induce contractions without increases in the intracellular calcium (Itoh et al., 1993). The phorbol ester, PDB, induced contraction in our experimental conditions in rat aorta strips. This functional evidence suggests the participation of protein kinase C in rat aorta contraction. Similar findings have been reported to occur in other vascular smooth muscles (Salaices et al., 1990). Furthermore, incubation during 2 h with PDB decreased the magnitude of successive contractions to UTP and its own contraction due to downregulation of the responses. The evidence of protein kinase C involvement in UTP-induced contractions is reinforced by the antagonism of the contractions produced by H-7 and staurosporine, and, in addition, these drugs relaxed the tonic contraction elicited by UTP. These tonic contractions to UTP are more sensitive to the protein kinase C inhibitors than to intracellular Ca^{2+}- and calmodulin-modifying agents, TMB-8 and W-7. However, in other vascular smooth muscle cells phorbol esters, but not staurosporine or H-7, decreased the UTP effect (Sánchez-Fernández et al., 1993). This controversy might be related to the existence of different P2Y receptors.

In summary, the pertussis-toxin-sensitive UTP-induced contraction in isolated rat aorta involves the release of intracellular Ca^{2+} and the activation of protein kinase C, presumably linked to phospholipase C.

Acknowledgements

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