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Short communication

Effects of α-trinositol administered extra- and intracellularly (using liposomes) on rat aorta rings

Eugen Brailoiu, Ovidiu Baltatu, Marcel Costuleanu, Sebastian Slatineanu, Catalin M. Filipeanu, Dimitrie D. Branisteanu *

Department of Physiology, University of Medicine and Pharmacy 'Gr. T. Popa', 16 Universitatii Street, Iasi, RO-6600, Romania

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Abstract

The effects of α-trinositol, a D-*myo*-inositol [1,2,6]trisphosphate derivative, were studied on de-endothelised rat aorta rings. The substance was applied extracellularly as well as intracellularly (by using liposomes as drug carriers). Upon extracellular administration, the drug reduced the level of contraction induced by 40 mM K^+ or by phenylephrine (10^{-5} M). No effects were observed on relaxed preparations. Liposomes containing α-trinositol induced a dose-dependent contraction of the preparations under resting tension with a threshold of 10^{-5} M in the aqueous phase. These contractions were heparin-insensitive but were significantly blocked by D-600 (10^{-5} M) (an L-type Ca^{2+} channel blocker) or in Ca^{2+}-free medium. Our data suggest that α-trinositol has a plasmalemmal mechanism of action which could involve Ca^{2+} influx from the extracellular space.

Keywords: α-Trinositol (D-*myo*-inositol 1,2,6-trisphosphate); Liposome; Smooth muscle, aortic, vascular

1. Introduction

Many groups have investigated the inositol phosphate-calcium signalling system. The involvement of inositol trisphosphate formation is well documented for many functional processes such as muscle contraction, metabolism, cell secretion, differentiation, etc. (Putney and Bird, 1983).

Although the mechanisms of action and the metabolism of inositol trisphosphates are relatively well known, there are still some areas less well understood. In this work we studied the effects of a new derivative, D-*myo*-inositol 1,2,6-trisphosphate, under the name of α-trinositol (formerly PP56), on rat aortic smooth muscle. There is increasing evidence concerning its modulatory effects on cardiovascular functions (Edvinsson et al., 1990; Edvinsson and Adamsson, 1992; Sun et al., 1992; Schwieler and Hjemdahl, 1993), affecting regional and cardiac haemodynamics (Gardiner et al., 1994).

In order to obtain further information concerning its mechanism of action, the effects of α-trinositol, administered either extra- or intracellularly, on rat aorta rings were compared. For intracellular application, liposomes were used as drug delivery system (Brailoiu et al., 1993a,b).

2. Materials and methods

2.1. Liposome preparation

The liposomes used in this physiological study were prepared from egg phosphatidylcholine (type X-E; Sigma), 60 mg lipid per ml of solution to be incorporated, according to the method described by Bangham et al. (1965) and modified by us (Brailoiu et al., 1993a,b).

Control liposomes contained only KCl (140 mM) (pH adjusted at 6.9). The same solutions were used to prepare liposomes containing α-trinositol (10^{-5} up to 10^{-2} M of α-trinositol in the hydration medium-aqueous phase) or heparin (5×10^{-5} M) (Sigma)-loaded liposomes.

The liposomes containing α-trinositol were administered in 2-ml volumes added to the 10-ml bath containing 8 ml of Krebs-Henseleit solution. The effects were

* Corresponding author. Fax +40-32-11 78 45.

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visible even for ratios of 0.2 ml of liposome solution/9.8 ml of Krebs-Henseleit solution but the best ratio was that first mentioned.

In order to remove the non-incorporated solutes, liposome batches were subjected to dialysis (dialysis sacks Sigma) in the Krebs-Henseleit solution (150 min, 1/600 v/v ratio, exchanging the saline buffer every 30 min). In order to control the quality of this procedure we dialysed, under the same conditions, all types of aqueous phases used for the preparation of liposomes. The control dialysis media were added, also in a 2/8 ml ratio in the organ bath.

2.2. Tissue preparation

To obtain the rat aortic rings, male Wistar rats (150–200 g body weight) were decapitated and exsanguinated. The thoracic aorta was rapidly removed and cut into rings, 2 mm wide. The endothelium was rubbed off gently with a smooth softwood stick. The rings were then mounted between hooks and their mechanical activity was monitored using an isometric force transducer and a potentiometric pen recorder (Linseis L650). The 10 ml organ bath contained Krebs-Henseleit solution (pH 7.4) with the following composition (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.6; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 5.5. In order to obtain the Ca^{2+}-free Krebs-Henseleit solution $CaCl_2$ was replaced (equimolar) by NaCl and EGTA 2 mM was added. The Krebs-Henseleit solution was kept at 37°C and aerated continuously with 95% O_2 + 5% CO_2. The resting tension was maintained at 2 g, the preparation being allowed to equilibrate for 2 h before starting the experiment. The lack of a functional endothelium was confirmed in each aortic preparation by its inability to relax in response to 10^{-5} M carbachol when precontracted by 40 mM K^+.

2.3. Electron microscopy technique

Electron micrographs of the liposome suspension were obtained by using a Tesla BS 500 electron microscope, after staining of the liposomes for 90 s in a phosphotungstic acid solution (1.5%).

2.4. Experimental protocols

Rat aorta rings prepared and isolated as described above, were used to investigate the effects of both modes of α-trinositol administration as follows: (a) the effects of α-trinositol (in concentrations between 10^{-10} and 10^{-2} M), administered extracellularly (added in the Krebs-Henseleit solution), upon the preparation under resting tension or precontracted with either 40 mM K^+ or 10^{-5} M phenylephrine; (b) the effects of α-trinositol (in concentrations between 10^{-5} and 10^{-2} M in the intraliposomal aqueous phase), administered by using liposomes, upon the preparation under resting tension.

2.5. Statistics

Student's t-test was used to identify statistically significant differences between groups (P values of less than 0.01 were considered significant) ($n = 6$).

3. Results

The vesicles had a diameter of 0.2–0.9 μm on the electron micrographs.

Extracellular administration of α-trinositol had no effect on the preparation under resting tension but significantly decreased the contractile force of preparations precontracted with 40 mM K^+ as well as phenylephrine (10^{-5} M) (Fig. 1).

Administered on relaxed preparations, control liposomes filled with 140 mM KCl had no effect while α-trinositol-loaded liposomes induced dose-dependent phasic contractions of rat aorta rings, with a 20–30 min plateau duration (Fig. 2b). Dose-effect curves were constructed taking into account the intraliposomal concentration of the drug. The maximal effect was found at 10^{-2} M inositol derivative dissolved in the aqueous phase (Fig. 2A).

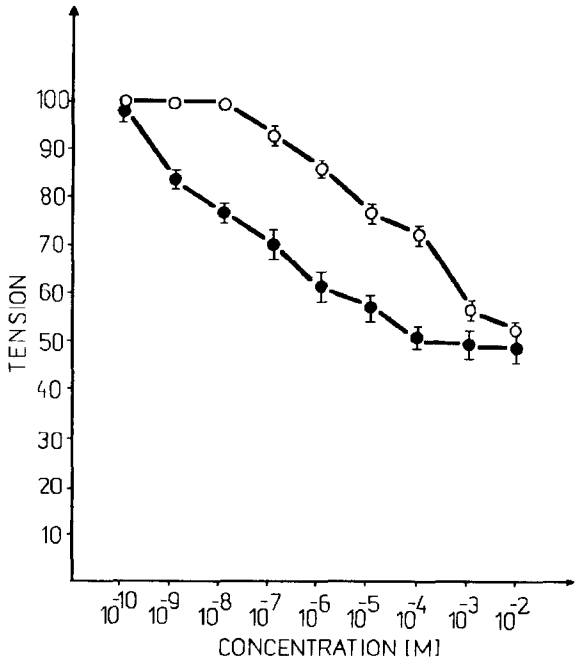

Fig. 1. Effects of α-trinositol, administered extracellularly, upon precontracted rat aorta rings (mean values ± S.E.M.). (●) precontraction induced by 40 mM K^+; (○) precontraction induced by 10^{-5} M phenylephrine.

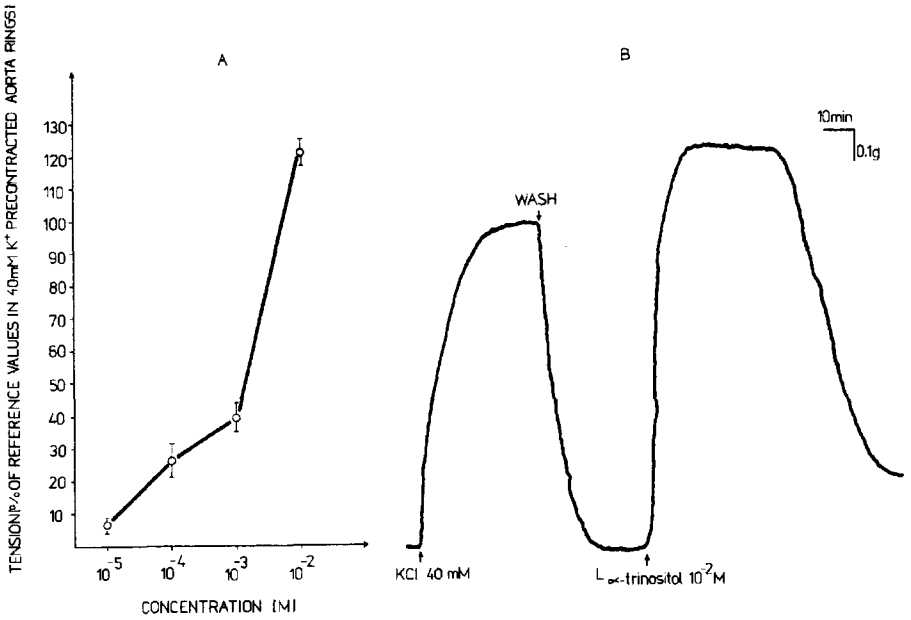

Fig. 2. A: Effects of α-trinositol (10^{-5} up to 10^{-2} M)-filled liposomes upon relaxed aortic rings (mean values ± S.E.M.). B: Effects of intraliposomal 10^{-2} M α-trinositol as compared to the control contraction induced by 40 mM K^+; actual record.

In Ca^{2+}-free Krebs-Henseleit solution, 10^{-5} to 10^{-2} M α-trinositol-filled liposomes had no effect upon the basal tone or upon the subsequent contractions elicited by phenylephrine 10^{-5} M.

Pretreatment (15 min) with D 600 (10^{-5} M), an L-type Ca^{2+} channel antagonist, inhibited the contractions induced by α-trinositol-loaded liposomes. Also, administration of D 600 (10^{-5} M) during the plateau (after 10 min) of the α-trinositol-induced contraction relaxed the aortic rings to the resting tension level.

In Ca^{2+}-containing solution, contractions elicited by α-trinositol liposomes were unaffected by pretreatment (15 min) with heparin (5×10^{-5} M)-filled liposomes. In addition, established contractions 10 min after administration of liposomes loaded with α-trinositol were unaffected by heparin-filled liposomes.

4. Discussion

α-Trinositol was shown to attenuate in mesenteric, gastroepiploic and uterine beds (Adamsson et al., 1992; Sun et al., 1992) or basilar artery (Wahlestedt et al., 1993) the neuropeptide Y- and ATP-induced pressor responses in a non-competitive way (Wahlestedt and Reis, 1993). In these type of arteries, α-trinositol has never been found to affect the vasoconstriction resulting from stimulation of α_1-adrenoreceptors or other phosphatidyl-inositide-coupled receptors (Wahlestedt and Reis, 1993). Moreover Gardiner et al. (1994) suggested that, at low doses, incremental infusions of α-trinositol in the rat do not reveal its full vasodilator potential, possibly due to concurrent activation of counter-regulatory vasoconstrictor mechanisms. The same authors also suggested that infusions of α-trinositol at a high dose substantially increase the renal, mesenteric and hindquarter blood flow and vascular conductances.

Our results indicate that, in an in vitro model (isolated and de-endothelized rat aorta rings), α-trinositol relaxed the contractions induced by depolarization (high K^+) as well as by the α_1-adrenoceptor agonist, phenylephrine. This difference could be explained by the different reactivities of vascular models as well as by the absence of endothelium (in our model). It has been shown that, when injected intravenously, α-trinositol has an anti-inflammatory effect (Claxson et al., 1990) in which endothelial cells may play a central role (Ziff, 1989).

The fact that, under resting tension, the extracellular administration of α-trinositol or control dialysis fluids had no effects suggests that the contractile response to liposomes containing α-trinositol could be induced by the drug delivered intracellularly.

The importance of Ca^{2+} in smooth muscle contraction is now clear. The fact that the α-trinositol-loaded liposomes contracted the rat aortic rings can be explained by an increase in intracellular Ca^{2+}. Use of liposomes kept the plasmalemmal barrier intact. Such an increase in intracellular Ca^{2+} induced by α-trinositol could be a consequence of either Ca^{2+} release from internal stores or an activated Ca^{2+} influx from the extracellular space, as already shown for other endogenous inositol phosphates (Berridge, 1993).

In contrast to what was found previously using D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] (Ghosh et al., 1988; Brailoiu et al., 1993b), the contractions evoked by α-trinositol were not affected by administration of liposome-entrapped heparin (5×10^{-5} M in aqueous phase; pretreatment or during plateau contraction). This suggests that α-trinositol might increase the cytosolic free Ca^{2+} through an Ins(1,4,5)P_3-independent mechanism which could be evoked either directly by α-trinositol or along secondary steps.

However, α-trinositol-loaded liposomes were unable to induce contraction in Ca^{2+}-free saline. Hence it is possible that the α-trinositol site of action could be localised at the plasmalemmal level as suggested by others (Yoo et al., 1994). It could activate, either directly or via another mechanism, a plasmalemmal Ca^{2+} influx. This hypothesis is also supported by the blocking effects of the D 600 pretreatment. This compound also relaxes the α-trinositol-precontracted vascular preparations. These data are also in agreement with the results obtained by Nelemans (1994) suggesting that, in cultured smooth muscle cells, intracellularly injected α-trinositol does not release Ca^{2+} from the intracellular stores.

In conclusion, our data suggest that α-trinositol could be an agent which, after intracellular administration, induces an increase of the cytoplasmic free Ca^{2+} concentration through a mechanism which could be different from that of Ins(1,4,5)P_3. This mechanism seems to involve plasmalemmal Ca^{2+} influx, either directly or through a secondary mechanism.

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