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Short communication

Effects of liposome-entrapped platelet-activating factor in the isolated rat trachea

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Abstract

The effects of platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine)-filled liposomes upon rat tracheal rings in vitro were examined. The capture of liposomes by the smooth muscle cells of the isolated tracheal rings as well as the release of their content into the cytoplasm was shown by using Evans blue (5×10^{-4} M)-loaded liposomes. Administration of PAF (10^{-3} M)-filled liposomes contracted the preparations, in contrast with extracellular administration of PAF and control liposomes, which had no effect. Administration during the plateau or pretreatment with liposomes containing BN 52021 (3-*t*-butylhexahydro-4,7*b*-trihydroxy-8-methyl-9*H*-1,7*a*-epoxymethano-1*H*,6*aH*-cyclopenta[*c*]furo(2,3-*b*)furo[3',2':3,4]cyclopental [1,2-*d*]furan-5,9,12(4*H*)-trione) ((10^{-3} M, a selective PAF receptor antagonist) or heparin (5×10^{-5} M) blocked this contraction. BN 52021 and heparin, not entrapped in liposomes, had no such effect. Our data suggest an intervention of PAF in the mechanisms of contraction of tracheal smooth muscle, involving a direct or indirect intervention (intracellular receptors for PAF cannot be excluded). At the same time, the rat trachea contraction induced by PAF-loaded liposomes could be linked to the PtdIns(1,4,5)P_3-dependent Ca^{2+} channels from the endoplasmic reticulum and/or to the interaction with G proteins, as shown by the blocking effects of heparin-containing liposomes.

Keywords: PAF (platelet-activating factor); Liposome; Smooth muscle, trachea

1. Introduction

Platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) acts via specific receptors on the membranes of responsive cells (Shukla, 1992). Recently, the PAF receptor-specific mRNA was detected in guinea pig lung, leukocytes, spleen and kidney (2.2, 3.0 and 4.0 kb mRNAs), but not in intestine, liver, heart or brain. In human tissues, the specific mRNA was found only in the placenta and lungs, but not in the heart, brain, liver, skeletal muscle, kidney, or pancreas. Some of these tissues that did not display a PAF receptor message are known nevertheless to respond to PAF (Venable et al., 1993). This discrepancy may support the findings of Hwang (1988) which suggested a second, possibly internal, PAF receptor. Some studies have already considered this possibility (Stewart et al., 1990).

Recently, liposomes have attracted interest as drug delivery systems for the respiratory system (Mayhew and Papahadjopoulos, 1983). One of the most interesting features of the experimental use of liposome-entrapped drugs is delivery of their content to the cytoplasm, avoiding contact with the outer surface of the cell. We thus tested the intracellular effects of PAF-loaded liposomes on the isolated trachea.

We demonstrated that the drugs could be delivered in the cytoplasm of tracheal smooth muscle cells by using Evans blue-filled liposomes and that the PAF-loaded liposomes contracted the isolated rat trachea.

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2. Materials and methods

2.1. Liposome preparation

The liposomes used in this study were prepared from egg phosphatidylcholine (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., 1993).

Control liposomes contained only KCl 140 mM solution (pH adjusted to 6.9). The same solution was used to prepare PAF (C16, Serva Feinbiochemica) (10^{-3} M)-containing liposomes, heparin (Sigma) (5×10^{-5} M)-loaded liposomes, BN 52021 (3-*t*-butylhexahydro-4,7*b*-trihydroxy-8-methyl-9*H*-1,7*a*-epoxymethano-1*H*, 6*aH*-cyclopenta[*c*]furo(2,3-*b*)furo[3′,2′:3,4]cyclopental [1,2-*d*]furan-5,9,12(4*H*)-trione) (Institut Henri Beaufour, Research Laboratories, Le Plessis Robinson, France) (10^{-3} M)-containing liposomes and Evans blue (5×10^{-4} M)-filled liposomes.

In order to remove the non-incorporated solutes all liposome batches were passed through a Sephadex G50 chromatography column (2 × 30 cm, Pharmacia Fine Chemicals).

2.2. Tissue preparation

To obtain the rat tracheal rings, male albino rats (150 g body weight) were killed by a blow on the head. The thoracic trachea was rapidly removed and cut into rings, 2 mm wide. Their epithelium was rubbed gently with a smooth softwood stick (randomly selected smooth muscle rings were examined histologically for confirmation of successful removal of the epithelium). The rings were then mounted between hooks and their mechanical activity was monitored using an isometric force transducer and a potentiometric pen recorder (Recorder OH-827, Radelkis, Budapest, Hungary and K 201, Karl Zeiss Jena, Germany). The 10-ml organ bath contained Krebs-Henseleit solution (pH 7.4) of the following composition (mM): NaCl, 118; KCl, 4.8; $MgSO_4$, 1.6; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 5.5; indomethacin (Sigma) 10^{-5} M. The perfusion buffer was kept at 37° C and aerated continuously with 95% O_2 + 5% CO_2. Resting tension was maintained at 2.5 g, the preparation being allowed to equilibrate for 2 h before the start of the experiment. The liposome suspension was added to the organ bath in volume ratios between 1/100 and 1/4.

2.3. Demonstration of capture of liposomes by tracheal smooth muscle cells

The encapsulation efficiency of liposomes containing Evans blue (5×10^{-4} M) was calculated using a spectroscopic method (EK1, Karl Zeiss Jena, Germany) (Brailoiu et al., 1993) at 611 nm (the absorbance wave length for Evans blue).

Using the method suggested by Hirnle (1991) the Evans blue-loaded liposomes were added to the organ bath (containing the mounted preparation and Krebs-Henseleit solution at 37° C, aerated continuously with

Fig. 1. Optical micrograph of tracheal smooth muscle cells. The blue cytoplasm (dark cells), in contrast with the rose (light cells), induced by eosin, indicates that Evans blue was delivered intracellularly by the means of liposomes. The bar corresponds to 100 µm.

95% O_2 and 5% CO_2) for 15 min in a 1/4 v/v ratio. For the usual optical micrographs we used standard eosin stain for paraffin sections.

2.4. Statistics

Responses to PAF, PAF-loaded liposomes, BN 52021, BN 52021-containing liposomes, heparin and heparin-filled liposomes were expressed as a percentage (mean ± S.E.M., $n = 6$) of the reference contractions obtained following the administration of carbachol 10^{-5} M.

We used Student's t-test to identify statistically significant differences between groups (P values less than 0.05 were considered significant).

3. Results

Removal of the epithelium was confirmed histologically. In the denuded preparations that were sampled, light microscopy demonstrated almost complete (≥ 90%) removal of the epithelium without obvious damage to the underlying submucosa or smooth muscle. No significant fluctuations in baseline tension were observed for epithelium-denuded preparations.

Administration of Evans blue-filled liposomes (with an encapsulation efficiency of 18.07 ± 0.42%) indicated that the drug was delivered in the cytoplasm of tracheal smooth muscle cells (Fig. 1).

The administration of non-liposomal PAF (extracellularly) in doses ranging from 10^{-10} M to 10^{-3} M had no effects on rat tracheal rings. Similar results were obtained with control liposomes (data not shown). In contrast, PAF (10^{-3} M)-loaded liposomes contracted the preparations to a level representing 81 ± 5.3% ($n = 6$) of the carbachol (10^{-5} M)-induced contraction. The above-mentioned results, which were maximal, corresponded to the direct addition of 2 ml liposome suspension to the 8 ml Krebs-Henseleit solution in the organ bath (1/4 v/v ratio). The addition of greater amounts of liposome suspension did not elicit stronger contractions. On the other hand, contractile effects appeared at a 0.1/1 v/v liposome suspension/Krebs-Henseleit solution ratio (7.6 ± 0.23%; $n = 6$). Furthermore, the contractile effects of PAF (10^{-3} M)-loaded liposomes increased with increasing values of the v/v liposome suspension/Krebs-Henseleit solution ratio as follows: 9.4 ± 0.35% at 0.125/1 v/v; 16.49 ± 0.75% at 0.15/1 v/v; 24.7 ± 0.62% at 0.175/1 v/v; 27.6 ± 0.98% at 0.2/1 v/v; 30.6 ± 0.87% at 0.225/1 v/v and 32.95 ± 1.13% at 1/4 v/v. These data show that the cumulative administration of increasing amounts of liposomes loaded with PAF (10^{-3} M) resulted in a maximal contraction (at 1/4 v/v) which is smaller by 59.32% than that obtained with the direct administration of the same total amount of PAF-loaded liposomes.

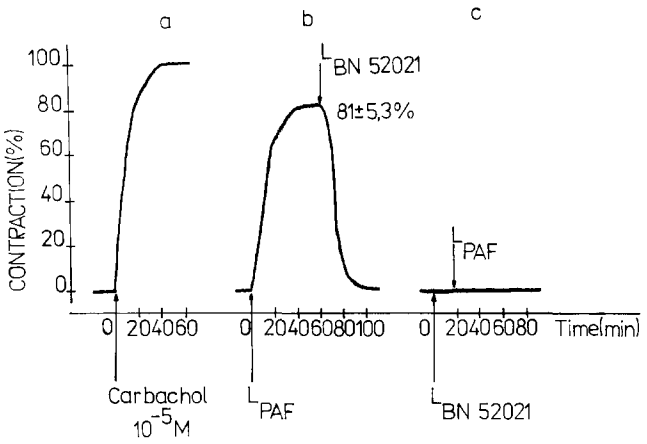

Fig. 2. Actual recordings. Trace a: Control contraction induced by 10^{-5} M carbachol. Trace b: Contraction induced by intraliposomal PAF (10^{-3} M) (81 ± 5.3% from the control contraction) and the effect of BN 52021 (10^{-3} M)-containing liposomes administered during the plateau of contraction induced by PAF-loaded liposomes. Trace c: Effect of BN 52021 (10^{-3} M)-filled liposomes administered as pretreatment (15 min) to the contraction induced by PAF-containing liposomes.

These contractions reached in 40 min a stable maximum tension level, which was maintained for at least 45 min thereafter, were totally reversible and blocked by administration, during the plateau (at 15 min of stable tension level) or as pretreatment (15 min prior to the PAF-loaded liposomes administration), of BN 52021 (10^{-3} M)-loaded liposomes (Fig. 2).

Similar results were obtained with the administration of heparin (5×10^{-5} M intraliposomal heparin 1/4 v/v ratio) as pretreatment or during the plateau of the contractions induced by PAF-containing liposomes (data not shown).

BN 52021 (10^{-3} M) and heparin (5×10^{-5} M) not entrapped in liposomes had no effect on the PAF (10^{-3} M)-containing liposomes-induced contractions, whether administered as pretreatment or during the plateau. Administration of BN 52021 (10^{-3} M)- and heparin (5×10^{-5} M)-filled liposomes as pretreatment or during the plateau of the carbachol (10^{-5} M)-induced contraction had no effects (data not shown).

4. Discussion

The presence of specific PAF receptors on the external surface of the cell membrane was recognized long ago in the rat trachea (Hwang et al., 1983). Despite this, in agreement with Prancan et al. (1982), our data show that free PAF does not contract the isolated rat trachea. The lack of concentration-effect

relationship could be linked to: (1) the continuous presence of indomethacin (10^{-5} M) added to the buffer; or, (2) the extremely low number of PAF receptors (even as few as 150 sites/cell) (Hwang et al., 1983); or, (3) PAF molecular and receptor heterogeneity: the PAF structure used in our experiments could be not the physiological specific ligand for the membrane receptor of rat tracheal smooth muscle cells; or, (4) the lack of platelets during the experiments; or, (5) the absence of albumin in the buffer; or, (6) the rapid metabolism of exogenous PAF.

Since control liposomes had no effect on the contractile activity of tracheal preparations, we can consider that the effects induced by PAF-loaded liposomes are the result of the transfer of the bioactive intraliposomal content to the interior of the smooth muscle cells.

Several studies have demonstrated that not all the effects of extracellular PAF are mediated by interaction of PAF with specific membrane receptors. PAF may have a role as an intracellular messenger and could elicit its effects through interaction with intracellular receptors (Marcheselli et al., 1990; Shukla, 1992). In our experiments, the effect of BN 52021, a selective PAF receptor antagonist, isolated by Braquet et al. (1985), to block the contractions induced by PAF-loaded liposomes could be interpreted either as possible evidence for an intracellular receptor of PAF or as a direct effect of BN 52021-containing liposomes, unrelated to PAF receptors. Since BN 52021-loaded liposomes did not affect the contractions induced by carbachol 10^{-5} M, we can consider that this blocking agent does not interact with the final contractile mechanisms.

Interaction of PAF with its specific receptor activates multiple biochemical pathways and several of these mechanisms may coexist (Shukla, 1992), including activation of phospholipid turnover (via phospholipases C, D and/or A_2) and G protein-coupled receptors. The inhibitory effect of liposome-loaded heparin on the contractions induced by PAF-containing liposomes is most probably due to the capacity of heparin to block the PtdIns(1,4,5)P_3-dependent Ca^{2+} release from the endoplasmic reticulum (Pyne and Pyne, 1993). However, its interaction with G proteins could also be somehow taken into account (Kobayashi et al., 1988).

Our data show that, in tracheal smooth muscle, PAF-filled liposomes have a contractile effect, which could be prevented by both BN 52021-containing liposomes and by heparin-loaded liposomes. Finally, the results suggest the existence of intracellular PAF receptors in rat tracheal smooth muscle cells and that the rat tracheal contractions induced by liposome-entrapped PAF could be linked to the PtdIns(1,4,5)P_3-dependent Ca^{2+} release from the endoplasmic reticulum and/or to the interaction with G proteins.

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