Interaction of synthetic peptides from annexin I and uteroglobin with lipid monolayers and their effect on phospholipase A_2 activity

RICHARD H. NEWMAN,* PAUL S. FREEMONT,† GEOFFREY J. BARTON,‡ and MICHAEL J. CRUMPTON*

**Cell Surface Biochemistry Laboratory and †Protein Structure Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, U.K. and ‡Laboratory of Molecular Biophysics, University of Oxford, Oxford OX1 3QU, U.K.*

Annexin I (lipocortin I) and uteroglobin are proteins which have been reported to have an inhibitory effect on phospholipase A_2 (PLA_2). Annexin I is a member of a family of at least eight different proteins which are known to bind to lipid membranes in a Ca^{2+}-dependent manner [1]. It has been suggested that the mechanism of annexin inhibitory action is by direct protein–protein interaction [2] although Davidson *et al.* [3] reported that annexin II (lipocortin II) and annexin I inhibit porcine pancreatic PLA_2 ($PPLA_2$) in the presence of Ca^{2+} by substrate sequestration. In both cases [2, 3] inhibition was completely overcome at higher substrate concentrations. Annexin I also inhibited partially purified preparations of two intracellular PLA_2s isolated from rat liver mitochondria and rat platelets, but again the inhibition decreased with increasing substrate concentration [4, 5]. From these results it was implied that annexin I interacts with the substrate rather than with the enzyme. Uteroglobin, a progesterone-binding protein with some claimed structural homology with PLA_2 [6] has been shown to have PLA_2 inhibitory properties [7] by binding directly to the enzyme.

More recently, experiments with synthetic oligopeptides corresponding to a region of purportedly high sequence similarity between annexin I (residues 246–254) and uteroglobin (the nine *C*-terminal amino acid of a α-helix 3) showed that these peptides have potent PLA_2 inhibitory activity *in vitro* and anti-inflammatory effects *in vivo* [8]. The authors suggested that the inhibitory effect of the peptide was executed through an interaction with the enzyme rather than with the substrate. However, van Binsbergen *et al.* [9], in a number of different PLA_2 assays *in vitro* have found no evidence for inhibition by these peptides, either by preincubation with the enzyme or by direct interaction with phospholipids.

Here we use a lipid-monolayer system similar to that described by Verger *et al.* [10] to examine both the ability of synthetic nonapeptides from annexin I and uteroglobin to interact with a lipid monolayer and to inhibit PLA_2 lipid hydrolysis. Synthetic peptides from annexin I, residues 246–254 (HDMNKVLDL), and from uteroglobin, residues 39–47 (MQMKKVLDS), as described above, were synthesized using a standard Fmoc solid phase approach with an automated synthesizer [11] and their sequences confirmed by amino acid sequence analysis.

Lipid monolayer experiments were performed to see whether the peptides were able to penetrate the lipid substrate, 1,2-didodecanoyl-*sn*-glycero-phosphorylcholine, as judged by their ability to cause an increase in surface pressure of the lipid monolayer at constant surface area. The annexin I peptide was incorporated into the lipid film as shown by the increase in surface pressure (Fig. 1*a*) of the lipid monolayer. The pressure at which the peptide was unable to penetrate the lipid film (critical surface pressure) was determined by extrapolating to zero surface pressure

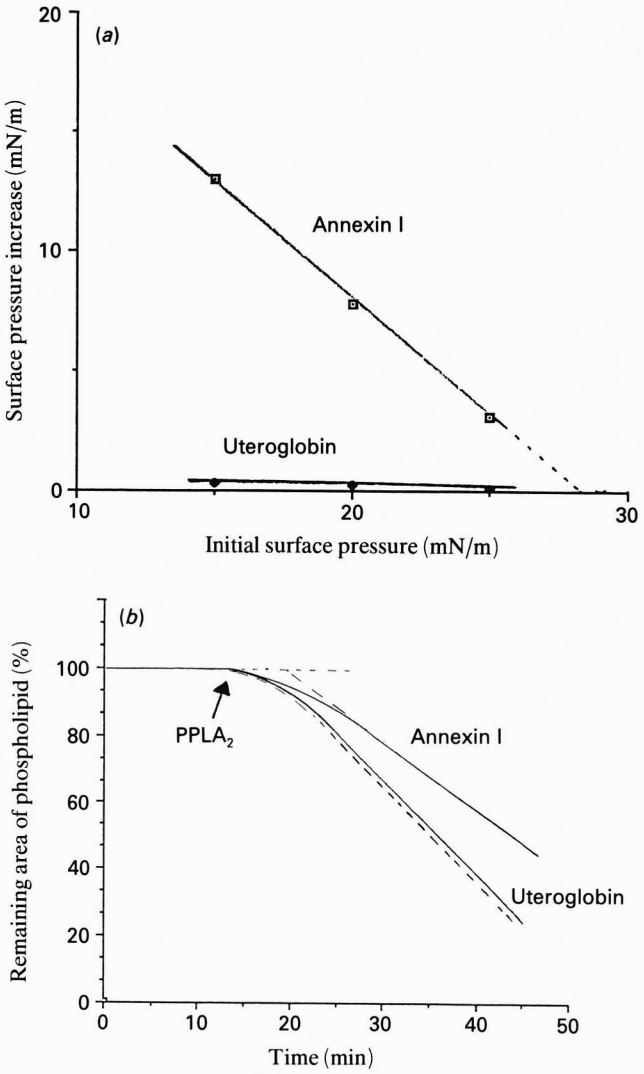

Fig. 1. *Effect of synthetic peptides on surface pressure and PLA_2 activity*

(*a*) Increase of surface pressure relative to the initial surface pressure of monolayer lipid after injection of 0.5 mg of synthetic peptide from annexin I or uteroglobin. (*b*) Enzymic velocity measured using a 15 mN/m lipid monolayer after injection of 28 ng of porcine $PPLA_2$ in the absence (−) and presence of the above peptides. The lipid monolayer used was 1,2-didodecanoyl-*sn*-glycero-phosphorylcholine and the buffer was 100 mM-Tris-HCl, pH 8.0, containing 20 mM-NaCl and 1 mM-$CaCl_2$.

increase and is 28 mN/m. In the case of the uteroglobin peptide no increase in surface pressure was detected. Further studies showed that the presence of the annexin I peptide in the lipid monolayer acted to inhibit the activity of $PPLA_2$ when the surface pressure of the film did not exceed 28 mN/m. Above this pressure the peptide no longer penetrated the lipid and inhibition of the enzyme could not be detected (data not shown).

Enzymic velocity measurements were made by injecting $PPLA_2$ under the lipid monolayer and recording the rate of

Abbreviations used: PLA_2, phospholipase A_2; $PPLA_2$, pancreatic PLA_2.

substrate hydrolysis as measured by the decrease in surface area at constant surface pressure, in the presence or absence of peptide (Fig. 1*b*). Enzymic velocity depended on the amount of enzyme at the interface and was seen to increase with time until it seemed to approach an asymptotic limit. In the absence of peptide the enzymic velocity at a lipid surface pressure of 15 mN/m was 11 mm/min per μg protein injected (0.30 nM). Preincubation of 0.5 mg of the annexin I peptide (4.0 μM) with the lipid monolayer resulted in a reduced enzymic velocity (7 mm/min per μg $PPLA_2$). However, no reduction in velocity was detected when the uteroglobin peptide was used (Fig. 1*b*) i.e. there was no detectable inhibition.

In conclusion, we have demonstrated that there is a difference in the way that the above two synthetic peptides interact with lipid films. One of them (from annexin I) was able to penetrate the lipid monolayer at pressures up to 28 mN/m, whilst the peptide from uteroglobin did not. Furthermore, using an enzyme kinetic assay we have shown that the annexin I synthetic peptide inhibited $PPLA_2$ activity at a monolayer surface pressure of 15 mN/m, whilst the peptide from uteroglobin had no significant inhibitory activity.

The above findings support those of van Binsbergen *et al.* [9]. These authors also tested the two nonapeptides as inhibitors of $PPLA_2$ and did not find inhibition at monolayer surface pressures of 30 mN/m. We have shown that when there is penetration of the lipid film as in the case of the annexin I synthetic peptide, then there is also an associated inhibitory effect on $PPLA_2$.

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Localization of lipocortin-1 in normal rat brain

PAUL STRIJBOS,* FRED TILDERS,† FRANK CAREY,‡ ROBERT FORDER‡ and NANCY ROTHWELL*

**Department of Physiological Sciences, University of Manchester, M13 9PT Manchester, U.K., †Department of Pharmacology, Free University, Amsterdam, The Netherlands, and ‡Bioscience 2, I.C.I.-Pharmaceuticals, Macclesfield, Cheshire SK10 4TG, U.K.*

Corticosteroids are potent anti-inflammatory drugs frequently used in the treatment of inflammation and disease. Their mode of action is multifactoral but in general requires the transcription of specific genes and subsequent formation of new proteins. One such steroid-inducible protein is lipocortin-1. Lipocortin-1 inhibits phospholipase A_2 activity in assays *in vitro* and thus supresses the generation of phospholipid-derived inflammatory mediators, including prostaglandins, leukotrienes and thromboxanes. Studies *in vivo* have shown that purified lipocortin-1 is able to inhibit both cytokine-induced fever and thermogenesis [1] and carrageenan-induced paw oedema [2]. We have recently published data suggesting a role for lipocortin-1 in the central effects of glucocorticoids [1]. Intracerebroventricular injection in conscious rats of a specific antiserum raised to a lipocortin-1 fragment antagonized the suppressive action of dexamethasone on interleukin-1β-induced thermogenesis and pyrogenesis. Recent immunohistochemical studies have demonstrated lipocortin-1 immunoreactivity in both normal and malignant human brain tissue [3] and rat peripheral tissues, but not in rat brain [4]. We have therefore undertaken an immunohistochemical study investigating the distribution of lipocortin-1 in normal rat brain, using a specific antiserum raised in rabbit to amino acids 1–188 of human lipocortin-1. Preliminary experiments have demonstrated that this antibody can recognize rat hypothalamic lipocortin-1 (F. Carey, R. Forder, N. Rothwell & P. Strijbos, unpublished work). Immunoreactive lipocortin-1 was visualized using a fluorescent isothiocyanate-labelled goat anti-rabbit antibody. Extensive lipocortin-1 immunostaining was observed in ependymal cells lining the lateral and third ventricle. Certain circumventricular organs, e.g. subcommisural organ, subfornical organ and the organum vasculosum of the lamina terminalis, contain specialized ependymocytes, and stained heavily for lipocortin-1. Another population of lipocortin-1-positive specialized ependymocytes is located in the floor and wall of the third ventricle. This population is called tanycytes (for review see [5]). Of these, only the lateral tanycytes are lipocortin-1 positive. The apical pole of these elongated cells contact the third ventricle and their processes arch ventrolaterally. They make contacts with capillaries and certain neuronal cells within the ventromedial hypothalamus, and with the capillary plexus in the lateral part of the median eminence. The topography and morphology of these lipocortin-1 immunoreactive tanycytes suggests that they enable blood-cerebrospinal fluid contact, although the role of lipocortin in these secretory processes remains unknown. In addition, a network of varicose fibres was found in the median eminence indicating that certain neurons may contain immunoreactive lipocortin-1. A low density of positive varicose fibers can be observed throughout the brain. Incidental positive staining cell bodies are present in the ventral hypothalamus. Lipocortin-1 immunoreactivity was also observed in pyramidal cells in the CA_2 and CA_3 regions of the hippocampus. This pattern showed similarities to the pattern of distribution of the interleukin-1, glucocorticoid and nerve growth factor receptor.

The present study has demonstrated lipocortin-1 immunoreactivity in distinct cells of normal rat brain and suggests that lipocortin-1 may participate in the secretory processes of tanycytes. Furthermore, its localization in nerve terminals suggest that lipocortin-1 may act as a signal protein