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.

Anaxin 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$_2$s 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 progestrone-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 a-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. 1a) 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 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$_2$.
Localization of lipocortin-1 in normal rat brain

PAUL STRIBOS,* 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, L.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 multifactorial 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 A2 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 antisem 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 antisem 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. Stribos, unpublished work). Immunooreactive 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 terminals, 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 fibres 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 CA1 and CA3 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 of lipocortin-1 synthetically peptide, then there is also an associated inhibitory effect on PPLA2.