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Interactions of chromogranin A-derived vasostatins and monolayers of phosphatidylserine, phosphatidylcholine and phosphatidylethanolamine

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Abstract

Vasostatin-I (CgA1-76) is a naturally occurring and biologically active N-terminal peptide derived from chromogranin A (CgA), produced and secreted at high concentrations by neuroendocrine tissues and also from a range of neuroendocrine tumors. This study aims to examine the hypothesis that in the absence of classical protein receptors CgA1-76 may, like its two derived peptides CgA1-40 and CgA47-66, perturb the lipid microenvironment of other membrane receptors, as a basis for the largely inhibitory activities of these CgA peptides. The nature of the interactions between phospholipids and vasostatin-derived fragments was studied in the Langmuir film balance apparatus at 37 °C. The synthetic peptides CgA1-40 and CgA47-66 and a recombinant fragment (VS-I) containing vasostatin-I (Ser-Thr-Ala-CgA1-78) were compared for their effects on monolayers of phosphatidylcholine and phosphatidylethanolamine from pig brain and defined species of phosphatidylserine. Marked differences in surface pressure–area isotherms and phase-transition plateaus were apparent with the three classes of phospholipids on VS-I, CgA1-40 and CgA47-66 in physiological buffer or pure water. The results indicate that VS-I and CgA47-66 at 5–10 nM concentrations may engage in electrostatic as well as hydrophobic interactions with membrane-relevant phospholipids at physiological conditions, VS-I in particular enhancing the fluidity of saturated species of phosphatidylserine.

Keywords: Recombinant human CgA1-78; CgA1-40; CgA47-66; Surface pressure–area isotherms; Increased fluidity; Langmuir apparatus

1. Introduction

Vasostatin-I is a major, naturally occurring N-terminal peptide derived from chromogranin A (CgA) which is a secretory product of the diffuse neuroendocrine system [1]. A wide range of biological activities, most of them inhibitory, has been assigned to vasostatin-I (CgA1-76), comprising two amphiphilic domains, i.e. CgA1-40 and CgA47-66. For example, vasostatin-I may inhibit parathyroid hormone secretion at low plasma Ca^{2+} [2] and vascular tone in bovine resistance arteries [3,4]. Negative inotropy has been demonstrated in three models of the working vertebrate myocardium (eel, frog and rat), exerted by the recombinant human Ser-Thr-Ala-CgA1-78 (VS-I), the synthetic peptides CgA7-57 and CgA1-40 under basal and stimulated conditions [5,6]. Moreover, VS-I contains an inhibitory activity against the TNFα induced extravasation in mice in vivo and gap formation in monolayers of human venous [7] and bovine arterial [8] endothelial cells in vitro. Stimulatory proadhesive effects by the CgA 47-64 region of VS-I have, on the other hand, been demonstrated for fibroblasts and vascular smooth muscle cells [9,10]. Finally, the naturally derived vasostatin-I, as well as the negatively charged CgA1-40 and the positively charged CgA47-66 (chromofungin) domains, exhibits potent antifungal activities associated with penetration into the fungi [11–13].

So far, classical, high affinity receptors have not been identified for the vasostatin derived peptides although specific membrane binding sites with K_d~40 nM have been reported

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for CgA1-40 in the bovine parathyroid cells [14] and cultured calf aorta smooth muscle cells [15]. A role for the cell penetrating, antifungal vasostatin peptides in the innate immunity has been proposed [16], and the largely inhibitory effects in mammalian systems have been postulated to serve homeostatic purposes, protecting the organism against excessive stimulation [17]. Receptor-independent cell penetration into microbial and mammalian membranes has been observed for a series of cationic and amphipathic peptides, such as the antimicrobial peptides implicated in self-defense and innate immunity. When assessed by the monolayer technique for surface pressure changes [18], both the negatively charged CgA1-40 and the positively charged chromofungin interacted with monolayers of egg phosphatidylcholine (PC) at 20 mN/m at 20 °C, and the surface pressure was further increased in the presence of sterols [12,13], suggesting penetration into the outer leaflet of the plasma membrane, which is dominated by PC [19]. In want of classical protein receptors on the membrane surface, the vasostatin derived peptides might exert biological activity by interaction with membrane phospholipids, thereby perturbing the structural environment necessary for proper function of other classical membrane receptors, analogous to that proposed for the cationic and amphiphilic drug chlorpromazine (CPZ) [20,21]. Whether the naturally occurring vasostatin-I, with its overall neutral charge and three amphipathic helical domains, interacts with other phospholipids (PL) of relevance for mammalian membranes at 37 °C, similar to or different from that of CgA1-40 and CgA47-66 in the presence of PC at 20 °C, remains to be established.

Cationic, hydrophobic and amphiphilic agents such as the antipsychotic phenothiazines have been named as modulators of multidrug resistance in cancer therapy, reflecting their ability to enhance the passive diffusion into the cancerous cells [22,23]. Analogously, the antipsychotic effects of CPZ, one of the phenothiazines, have in part been ascribed to electrostatic interaction with acidic PL, notably with phosphatidylserine (PS), as demonstrated in model experiments by the Langmuir monolayer technique [20,21].

The neutral PC and phosphatidylethanolamine (PE) and the acidic PS are asymmetrically distributed over the plasma membrane, with PC on the outside and PS and PE on the inside [18]. This asymmetry is essential for upkeep of critical signal transduction cascades, cell shape, haemostasis and homeostasis [24]. A collapse of this asymmetry leads to enhanced exposure of PS on the cell surface and occurs as a general feature of senescence and apoptosis, as evident in aging erythrocytes, platelets and tumor cells [25,26]. For instance, anionic lipids expressed on activated platelets are indispensable in promoting membrane binding and the catalytic activity of the complexes that lead to the formation of thrombin and deposition of the clot-forming fibrin matrix [27].

The aim of the present study has been to explore the possibility of interactions between the amphipathic vasostatin-derived peptides and membrane PL as a receptor-independent pathway for modulation of cell signalling with defined monolayers of PC, PE and PS in order to expand our previous findings of interactions between monolayers of PC (egg lecithin) and subnanomolar concentrations of CgA47-66 [12] and CgA1-40 [13].

CgA circulates at supranormal concentrations (>2 nM) in patients with various tumors of neuroendocrine origin [28] and also in non-cancerous, inflammatory and pathological states such as chronic heart failure [29,30]. Here, we compare the nature of interactions of low nanomolar concentrations of VS-I and its derivatives, CgA1-40 and CgA47-66, with defined monolayers of PC, PE and PS, expressed by surface pressure–area isotherms as recorded in the Langmuir film balance apparatus at 37 °C. By this technique a number of parameters such as the nature and the packing of lipid molecules, the composition of the subphase and the temperature may be varied in a definite way to distinguish between details in the pH, ionic composition, length and saturation of the acyl chains.

2. Materials and methods

2.1. Measurements of surface pressure–molecular area isotherms

Surface pressure–area isotherms are important indicators of the monolayer properties of an amphiphilic material and may be obtained from measurements of the surface pressure as a function of the area of water surface available to each molecule. In this study the isotherms were obtained as described by Agasøster and Holmsen [20], using a Langmuir apparatus KSV mini trough (Helsinki, Finland) of dimension $75 \times 365 \times 5$ mm ($W \times L \times H$) and coated with polytetrafluorethylene to prevent any leakage of the subphase over the edges. The trough was thermostatted by circulating water in channels placed underneath the Teflon trough. The surface area of the trough was measured by sweeping movable barriers over the surface of the trough. The barriers were coated with polyacetal Delrin, a hydrophilic material, and heavy enough to prevent any leakage of the monolayer beneath the barrier. The barriers were driven symmetrically along the long side of the trough by a motor controlled by the KSV minitrough software. The trough and barriers were carefully cleaned after each run three times with 96% ethanol and distilled water. A volume of 15 μl of PL in chloroform (1 mg/ml) was carefully spread with a Hamilton syringe on the surface of the subphase consisting of Krebs Ringer phosphate buffer (KRP) or pure Milli-Q water. Thereafter the compression of the barriers was started. The film was compressed by reducing the surface area by means of the barriers at a constant rate for 20–30 min while continuously monitoring the surface pressure by the Wilhelmy plate method. The Wilhelmy plate consisted of a 10×20 mm plate of platinum, connected to an electrobalance above the trough, and immersed about 5 mm into the subphase. Changes in the composition of the liquid surface caused changes in the force working on the plate. The forces were then converted into surface pressure (mN/m), taking into account the dimensions of the plate. Recordings of the surface pressure (mN/m) against mean molecular area ($Å^2$) were performed by means of a computer. The amount of applied lipid and its molecular