Interaction of the N-terminal domain of chromogranin A (vasostatin derived peptides) with the rat posterior cerebral artery.

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Chromogranin A (CgA), an acidic granule protein of the regulated secretory pathway in the diffuse neuroendocrine system, is postulated to serve as a prohormone for vasoactive peptides derived from the hydrophobic N-terminal domain¹. In rat, the first cleavage product is betagranin (rCgA₁₋₁₂₉), in which the first 76 amino acid residues are 86 % homologous to the bovine vasostatin I (bCgA₁₋₇₆), previously shown to be vasoinhibitory in human and bovine blood vessels². The aim of the present study has been to characterize interactions of vasostatin I with rat posterior cerebral arteries (rPCA). Inverted fluorescence microscopy was applied to show the interaction of the rhodamine-labeled bCgA-derived peptides: bCgA₇₋₄₀ and bCgA₄₇₋₇₀ with the adventitial and luminal structures of rPCA. The results are consistent with a specific interaction of bCgA₄₇₋₇₀ with the smooth muscle cells but not with the endothelial cells.

MATERIALS AND METHOD

rPCA were removed under a dissecting microscope from the brain of male Wistar Kyoto rats (14 - 16 weeks of age). Segments of the isolated artery were loaded from the luminal or adventitial side with rhodamine (Rh)-labeled peptide in HEPES-PBS for 2 hours at room temperature. In a parallel series arteries were mechanically denuded using a fine metal wire prior to luminal peptide loading. After, completed loading the vessels were thoroughly rinsed with HEPES-PBS and placed on a cover glass in a drop of HEPES-PBS for analysis with an inverted fluorescence microscope.

RESULTS

Incubation of rPCA with peptide-free rhodamine $(1 \ \mu M)$ resulted in an intensely fluorescent artery, whether added to the adventitial or the luminal side. Adventitial loading with Rh-CgA ₇₋₄₀ 1 μ M did not give any fluorescence labeling of the artery. Loading with Rh-CgA₄₇₋₇₀ revealed on the other hand a strong arterial fluorescence at both 1 nM and 1 μ M peptide concentration. Luminal loading with Rh-labeled peptide failed to stain the artery, whether exposed to 1 μ M Rh-CgA₇₋₄₀ or 1 μ M Rh-CgA₄₇₋₇₀. In denuded arteries luminal loading with 1 μ M Rh-CgA₄₇₋₇₀ resulted in an intense fluorescence, this was not observed with 1 μ M Rh-CgA₇₋₄₀.

The endothelial lining of the rat cerebral artery did not interact with rhodamine-labeled N- and C-terminal peptides of vasostatin I. In contrast to the N-terminal peptide CgA_{7-40} , the cationic C-terminal peptide Rh-CgA₄₇₋₇₀ interacted with the rat vascular smooth muscle cells at a functionally relevant concentration (1nM). This suggests a role for the cationic domain of vasostatin I in regulation of smooth muscle function from the adventitial side. The positive charge of bCgA₄₇₋₇₀ (+2.5), as opposed to the negative charge of bCgA₇₋₄₀ (-2), might explain the selective interaction of CgA₄₇₋₇₀ with the rat artery.

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