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 domain[1]. In rat, the first cleavage product is betagranin ($rCgA_{1-129}$), in which the first 76 amino acid residues are 86 % homologous to the bovine vasostatin I ($bCgA_{1-76}$), previously shown to be vasoinhibitory in human and bovine blood vessels[2]. 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: $bCgA_{7-40}$ and $bCgA_{47-70}$ with the adventitial and luminal structures of rPCA. The results are consistent with a specific interaction of $bCgA_{47-70}$ 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 μM) resulted in an intensely fluorescent artery, whether added to the adventitial or the luminal side. Adventitial loading with Rh-CgA_{7-40} 1 μM did not give any fluorescence labeling of the artery. Loading with Rh-CgA_{47-70} revealed on the other hand a strong arterial fluorescence at both 1 nM and 1 μM peptide concentration. Luminal loading with Rh-labeled peptide failed to stain the artery, whether exposed to 1 μM Rh-CgA_{7-40} or 1 μM Rh-CgA_{47-70}. In denuded arteries luminal loading with 1 μM Rh-CgA_{47-70} resulted in an intense fluorescence, this was not observed with 1 μM Rh-CgA_{7-40}.

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-CgA_{47-70} 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 $bCgA_{47-70}$ (+2.5), as opposed to the negative charge of $bCgA_{7-40}$ (-2), might explain the selective interaction of CgA_{47-70} with the rat artery.

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