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# The Effect of Furosemide on GAG Alterations Induced by Bilateral Aortic Denervation in the Rat Aorta and Cerebellum

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Hypertension is a major risk factor leading to atherogenesis. We have recently shown that glycosaminoglycans (GAGs) of the arterial wall are essentially inovlved in processes leading to atherogenesis. GAGs also play the main structural role in brain morphology, the integrity of which has recently been shown to be affected by hypertension. The aim of this study was to investigate the effect of arterial hypertension, induced by bilateral aortic denervation (bAD) in rats, and of the subsequent antihypertensive treatment with furosemide (S.C., 3 mg, twice daily) on the synthesis of GAGs in the aorta and cerebellum. Male Wistar rats weighing 250-300 gr were subjected to bAD, by cervical approach under pentobarbital/ketamine (I.P.) anesthesia. Sham-operated animals received similar cervical incisions leaving nerves intact. Arterial blood pressure was measured by catheterization of the iliac artery. At days 1, 2 and 7 after surgery, animals were sacrificed and the abdominal aorta and cerebellum were collected. Samples were homogenized and the isolation of GAGs was performed by lipid extraction and extensive digestion with pronase and DNAse. Total glycans were purified from the digestion products by ethanol precipitation and quantified by measuring their uronic acid content colorimetrically. We observed that there were no signifcant changes in the total amount of uronic acids measured the cerebellum of bAD animals. However, the content of uronic acids in the aorta of bAD animals significantly decreased from day 1 to day 7 postoperatively, as compared to sham-operated animals. This decrease was alleviated in bAD animals treated with the antihypertensive agent furosemide. GAGs were further fractionated according to size and charge using electrophoresis on polyacrylamide gradient gels and cellulose acetate membranes, respectively. The nature of the GAG fractions was identified by enzymatic treatment with GAG-degrading enzymes. Major GAGs identified in the rat aorta as well as in the cerebellum were heparan sulfate, dermatan sulfate, chondroitin sulfates and hyaluronic acid. No significant changes were observed in the nature and content of the individual GAGs in the in aortas of bAD animals. In the cerebellum of bAD animals, at days 2 and 7 postoperatively, we observed a significant increase in the relative content of heparan sulfate, as comapred to sham-operated animals, accompanied by a concomitant decrease in the relative content of hyaluronic acid. The above described alterations in GAG synthesis in the cerebellum were not affected significantly in bAD animals treated with furosemide. These structural changes induced by arterial hypertension may be of high functional significance and may be associated with a protective mechanism accounting for the rare incidence of infarction in the territory of the superior cerebellar artery and in atherogenic processes in the aortic wall.