Development of immunoreactive atrial natriuretic peptide in fetal hearts of spontaneously hypertensive and Wistar-Kyoto rats

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Summary. The development of immunoreactive atrial natriuretic peptide (ANP) was studied in fetal hearts of spontaneously hypertensive (SHR) and compared to normotensive Wistar-Kyoto (WKY) rats. While SHR fetal hearts were noticeably less developed than those of WKY at 10 and 11 days of gestation, both strains showed ANP immunoreactive cells in some but not all primitive heart tubes. At 12 days additional ANP immunoreactive cells appeared in formative trabeculae of the ventricle and atrium. ANP cells were also observed in the myogenic layer of the truncus and bulbus arteriosus and their derivatives from 11 through 16 days, but not at 18 days. In both strains, there were more ANP cells in the left ventricle than in right beginning at day 13. There were no obvious strain differences in the developmental pattern and timing of ANP producing cells. However, on the day of birth, staining was reduced in hearts from some WKY newborn pups compared with hearts from SHR newborns and ventricular staining was reduced in both strains when compared to fetal hearts. These observations indicate that ANP is one of the earliest peptide hormones produced and that the predisposition to genetic hypertension does not appear to influence the development of ANP.

Key words: Atrial natriuretic peptide – Fetal heart – Spontaneously hypertensive rat – Immunohistochemistry

Introduction

In 1956, Kisch described dense granules in mammalian atrial cardiocytes which indicated that the contractile cells had a secretory function. Later DeBold (1979) demonstrated a relationship between the number of atrial granules and water-sodium intake and balance. It was shown that a large number of dense granules was released from the heart in response to salt intake. The content of the granules has now been well characterized to be a prohormone to a polypeptide hormone called atrial natriuretic peptide (ANP), cardioperatin, or atrial natriuretic factor (Forssmann et al. 1986). In the adult mammal, ANP regulates blood pressure and extracellular fluid volume by eliciting natriuresis, diuresis and vasodilation (Cantin and Genest 1987). With radioimmunoassay, Dolan and Dobrozsi (1986) found ANP in fetal rat hearts at 14 days of gestation. This was confirmed by immunohistochemical localization of ANP in atria and ventricles of fetal rat hearts at 20 days of gestation (Scott and Jennes 1987). The purpose of the present study was to compare the early developmental pattern of ANP in fetal hearts of spontaneously hypertensive rats (SHR) to those of Wistar-Kyoto (WKY) rats from just after the initial heart beat at about 9.5 days gestation until the day of birth.

Materials and methods

Fetuses of three pregnant SHR and three pregnant WKY rats were used for each day of gestation 10, 11, 12, 13, 16, and 18. Day 0 of gestation was defined as the day of insemination. Two or three entire fetuses (days 10 through 16) per day or two or three fetal (day 18) or newborn hearts per day were fixed in Bouin's fluid. To determine the day of birth, cages were checked in the morning and afternoon. Tissues were dehydrated in graded alcohols, cleared in xylene and embedded in Paraplast. Coronal or sagittal sections were cut at 7 μm and mounted on acid-clean, gelatin chrom-alum subbed slides. Sections were processed for immunohistochemistry with a specific rabbit antiserum to ANP (10-5) which was generated against the synthetic ANP, atriopeptin III (rat 5-28, Bachem), coupled to keyhole limpet hemocyanin via 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide HCl (Jennes and Stumpf 1983). The antisera recognizes the N-terminus of synthetic atriopeptin III as determined by radioimmunoassay. The primary antisera was used in a dilution of 1:1000 for 24 h at room temperature, followed by an incubation with sheep anti-rabbit IgG (1:100, Antibodies, Inc.) and rabbit peroxidase antiperoxidase complex (1:100, Sternberger-Meyer, Inc.). All antisera were diluted in Dulbecco’s phosphate buffered saline (0.1 M, pH 7.4) containing 1% normal Iamb serum and 0.1% sodium azide. The sections were stained with a fresh solution of 0.075% 3,3’ diaminobenzidine-4-HCl/Tris buffer (0.05 M, pH 7.6) and 7 μl H2O2 (30%) per 100 ml. Specificity controls for the immunohistochemical staining included incubation with preimmune serum...
Fig. 1a, b. SHR fetal heart at 10 days gestation. a Small mounds of cells in the myocardium indicate beginning trabeculae formation (T). b Punctate accumulations of immunostained ANP in the myocardium (arrowheads). a × 130, b × 770

Fig. 2a, b. WKY fetal heart at 10 days gestation. a Trabeculae have formed (T). b ANP positive cells are present in the trabeculae (arrowheads). a × 130, b × 770

Fig. 3a, b. WKY fetal heart at 11 days gestation. a A common cardinal vein (C) joins the sinu-atrial chamber (A) which is continuous with the ventricle (V). b ANP positive cells stop abruptly at the entrance of the venous return (arrowheads). a × 130, b × 770