70) Radioimmunoassay for Atrial Natriuretic Polypeptide. Akira Sugawara¹, Kazuwa Nakao^{1,2}, Narito Morii¹, Makoto Sakamoto¹, Mitsuaki Suda¹, Masahiro Kihara³, Yukio Yamori³, Masanori Shimokura⁴, Yoshiaki Kiso⁴, and Hiroo Imura¹.
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Mammalian atrial cardiocytes have spherical, electron-opaque granules (specific atrial granules), which show morphological resemblance to secretory granules observed in peptide hormone-producing cells. Recently, extracts from mammalian atria have been shown to contain multiple forms of natriuretic factors of peptide nature which cause natriuresis and diuresis when injected into rats.

Kangawa and Matsuo have reported that three bioactive components, α -, β -, γ -human atrial natriuretic polypeptide (hANP) with apparent molecular weights of 3K, 5K, 13K, respectively, are present in human atrial extracts, and determined the complete amino acid sequence of a 28-amino acid peptide, α -hANP, which is a major bioactive peptide (Biochem Biophys Res Commun 118: 131, 1984). From rat atrial extracts, Flynn et al have isolated a peptide with 28 amino acids which revealed a single amino acid replacement of methionine by isoleucine at the position 12, when compared with α -hANP (Biochem Biophys Res Commun 117: 859, 1983). In addition to this rat atrial peptide, α -rat ANP (α -rANP), other several peptides, both amino- and/or carboxy-terminally deleted forms and amino-terminally elongated forms of α -rANP, have been identified from rat atrial extracts.

In the present study, we have prepared an antiserum which can recognize equally α -hANP and α -rANP using a synthetic common carboxy-terminal fragment, α -hANP(17-28) or α -rANP(17-28), and established a sensitive and specific radioimmunoassay (RIA) for α -hANP and α -rANP.

The linear 28-amino acid peptides or reduced forms of α -hANP and α -rANP and carboxy-terminal fragments, α -ANP(17-28) and α -ANP(24-28), were synthesized by a conventional solution method. The reduced forms of α -hANP and α -rANP were then converted to the oxidized forms by air-oxidation under high dilution conditions. α -ANP(17-28) was conjugated to bovine thyroglobulin using the carbodiimide coupling procedure. Conjugated α -ANP(17-28)(100-200 μ g) was emulsified with an equal volume of Freund's complete adjuvant and used for immunizing Japanese white rabbits. Antisera were obtained after each booster injection. ¹²⁵I- α -hANP was prepared by the chloramine T method. The specific activity of ¹²⁵I- α -hANP ranged from 500 to 900 μ Ci/ μ g. Using the antiserum (CR-3) and ¹²⁵I- α -hANP, we have set up a sensitive and specific RIA for α -ANP. The RIA mixture was incubated for 48 hrs at 4°C. Bound and free ligands were separated by dextran-coated charcoal method.

The RIA could recognize α -hANP and α -rANP equally. Furthermore, the RIA also reacted equally with α -ANP(24-28), the carboxy-terminal five amino acids of α -ANP, and α -ANP on a molar basis. The significant inhibition of the binding of ¹²⁵I- α -hANP to the antiserum was observed with as little as 50 pg of α -ANP. The range for measurement of α -ANP was 50 pg to 10 ng/tube.

The dilution curves of extracts from human right auricles obtained at operation and rat atria were parallel to the standard curve of the RIA. Large amounts of α -ANP-like immunoreactivity (α -ANP-LI) were detected in these tissues. The concentrations of α -hANP- and α -rANP-LI in human right auricles and rat atria were 160 $\pm$ 29.5 μ g/g and 174 $\pm$ 14.1 μ g/g, respectively. The RIA could detect not only α -hANP- and α -rANP-LI in human and rat atria but also α -ANP-LI in dog and guinea pig atria.

These results suggest that the RIA for α -ANP established in the present study, which can recognize the carboxy-terminal portion of α -ANP, is a useful tool to investigate the distribution and the pathophysiological significance of α -ANP and its related peptides in cardiovascular disorders of various species including man and rat.