ATRIAL NATRIURETIC PEPTIDE (ANP) ASSAYED IN PLASMA OF SQUALUS ACANTHIAS

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The rectal gland of Squalus acanthias is stimulated to secrete by human and rat atriopeptins as well as by a peptide contained in crude extracts of shark heart. This suggests that a cardiac peptide constitutes the blood-borne signal that initiates rectal glandular salt secretion when intravascular volume is expanded. However, direct evidence for this hypothesis is lacking. The cardiac peptides native to the shark have not been isolated, nor are their structures known, and a specific antibody useful in their quantitative assessment has therefore not yet been developed.

The purpose of the present experiments was to see whether antisera already developed to atriopeptins of other species might detect changes in immunoreactivity in the blood of sharks, when vascular volume was acutely expanded so as to stimulate rectal gland secretion.

Six male specimens of Squalus acanthias, weighing 1-3 kg, immobilized by pithing, were studied by the technique of Solomon et al (Am J Physiol 1984; 246:R63-R66), in which an explanted rectal gland is perfused continuously with blood led from the aorta of a donor fish. Samples of blood from the donor fish were drawn from an inlying PE-90 catheter inserted in the dorsal aorta. The same catheter was used to infuse isotonic shark Ringer solution in order to produce volume expansion. Blood flow and secretory rate of the explanted gland were monitored by collection catheters inserted into its vein and excretory duct.

After an initial equilibration period of 30 minutes, during which basal measurements of glandular blood flow and secretory volume were obtained, 8 ml of blood were drawn from the dorsal aorta for determination of the basal level of ANP. Shark Ringer solution, 150 ml, was then infused over the next 20-30 minutes, while duct flow and blood flow to the gland were monitored for 60 minutes after the start of volume expansion. At the end of this time, a second 8 ml sample of blood was drawn for ANP level.

For determination of plasma ANP levels, 8 ml of blood were collected into iced plastic tubes containing 160 µl of 15% EDTA, and immediately centrifuged in the cold. Two to three ml of acidified plasma were extracted via Sep-pak C18 cartridges (Waters Associates, Milford, MA) with 15 ml 10 mM trifluoroacetic acid (TFA) and 2.5 ml of 60% acetonitrile in 10 mM TFA as the eluate. The extracts were then fast-frozen with acetone and dry ice and stored in polypropylene tubes at -70°C until assayed. All samples were lyophilized prior to assay and reconstituted in 250 ul of assay buffer (50 mM sodium phosphate, pH 7.4, containing 0.2% (w/v) bovine serum albumin, 10 mM EDTA, 0.1% (v/v) Triton X-100, and 0.01% sodium azide). Radioimmunoasay was performed using a double antibody technique with rabbit anti-human alpha-ANP (Amersham, UK).

Synthetic alpha-hANP, 28 amino acid (Peninsula Laboratories, Belmont, CA) was used to construct standard curves. The efficiency of the extraction procedure was estimated by recovery of purified synthetic radiolabelled ^{125}I alpha human ANP added to plasma before extraction. ANP added to human plasma has a recovery rate of 94.4%. Interassay variation is 22%. Intra-assay variation is 10%. Using this procedure the lowest concentration of alpha-hANP detected was 22 pg per tube or 8 pg/ml of plasma.

Volume expansion of the donor shark induced a substantial increase in blood flow to the explanted gland (11.1 + 2.1 to 47.3 + 6 ml/hr, mean $\pm$ s.e.) and in glandular secretion (0.52 + 0.12 to 1.8 + 0.3 ml/hr). ANP immunoreactivity in the plasma averaged 60.8 $\pm$ 12.5 pg/ml (expressed as human ANP equivalents) during the basal state, and rose by an average of 50 $\pm$ 20.8 ($p < 0.025$) after volume expansion. These levels are comparable to those measured in human subjects whose blood volume is expanded. The magnitude of the rise in ANP could not be correlated with the degree of rectal gland stimulation in these experiments.

The significance of the increase in ANP levels found after volume expansion with the Amersham anti-human ANP antibody is underlined by our failure to demonstrate an increase with other antisera raised against the ANP of mammalian species. These included anti-rat ANP (Peninsula Laboratories), anti-rat ANP (Amersham), and anti-human ANP (Peninsula Laboratories). This suggests that the shark cardiac hormone is sufficiently different in structure from rat and human cardiac peptides to be poorly reactive to most antisera specific to these molecules, but that there are enough congruities to permit cross-reaction with certain anti-ANP antibodies (e.g., Amersham anti-human). Such antibodies should prove useful in steps leading to the isolation and characterization of cardiac peptides native to Squalus and in elucidating their physiological function.

The increase in circulating immunoreactivity to an antiserum raised against human ANP, after volume expansion of Squalus acanthias, strengthens the hypothesis that a native cardiac peptide with an antigenic component resembling human ANP is secreted into the bloodstream, mediating the rectal gland response.

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