

RAT ATRIAL NATRIURETIC POLYPEPTIDE STIMULATION  
OF ADRENAL ZONA GLOMERULOSA CELL GROWTH

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Rat atrial natriuretic polypeptide (rANP) was found to stimulate [ $^3\text{H}$ ] thymidine incorporation into the DNA of bovine adrenal glomerulosa cells in a primary culture in a dose-dependent manner. The minimum effective dose was a very low concentration ( $10^{-12}$  M of ANP), suggesting that ANP had a physiological effect. These findings are the first indication that ANP possesses growth-stimulating activity with regard to adrenal zona glomerulosa cells. © 1985 Academic Press, Inc.

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Since atrial natriuretic polypeptides (ANPs) were isolated from the human(1) and rat atrium(2-4), extensive studies have been done to clarify their physiological roles. In addition to the kidney and vessels, the adrenal gland was also identified as an ANP target organ. Specific binding sites for rat ANP were found in bovine adrenal zona glomerulosa cells(5), and ANP was found to inhibit aldosterone production(5-7).

In this communication, we report another role of ANP in regard to adrenal zona glomerulosa cells, i.e., stimulation of cellular growth.

Materials and Methods

Bovine adrenal glands were obtained at a local slaughterhouse. After fat was removed, outer cortical cells (0.3 mm thickness from the capsule) were removed using a tissue slicer (Natsume Kogyo Co. Ltd., Tokyo). These outer slices, mainly composed of zona glomerulosa cells, were minced and treated enzymatically with Dispase (Mikuni Kagaku Co. Ltd., Tokyo) (1500 U/ml) at 20°C for 20 minutes. After filtration through nylon mesh (No. 100), cells were washed twice with Hanks' solution and resuspended in Ham's F-10 medium with 10% FCS. Aliquots of the suspended cells ( $10^5$ /ml) were plated in 24-well dishes (A/S Nunc, Denmark) and incubated at 37°C in 5% CO<sub>2</sub>-95% air. 18 hours after plating, the medium was removed and cells were incubated in Ham's F-10 alone for 72 hours. 10 hours after the addition of synthesized rat 1-28 ANP (Peptide Institute Inc., Osaka), [1,2-methyl- $^3\text{H}$ ] thymidine (101 Ci/mmol; New England Nuclear Corp., Boston, MA) was added to cultures at a final concentration of 1  $\mu\text{Ci}/\text{ml}$ . 12 hours later, cells were washed twice with cold Hanks' solution and incubated with

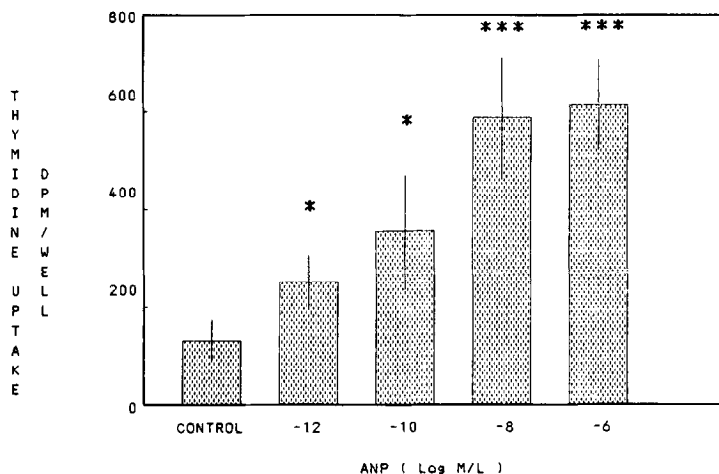


Fig. 1. Dose responses of ANP on zona glomerulosa cell growth. Each bar represents mean  $\pm$  SD (n=4). \*p<0.05 vs. Controls,\*\*\*p<0.001 vs. Controls.

10% trichloroacetic acid (TCA) for 15 minutes at 4°C. After subsequent washes with cold 10% TCA and ethanol-ether (3:1), precipitates were dissolved by the addition of 0.5 ml of 1N NaOH. The solution neutralized with 1N HCl was then moved to a scintillation vial with 5 ml of ACS II (Amarsham Corp., Ill.) and counted in a scintillation counter. Student's t test was used for statistical analysis.

### Results

As shown in Fig. 1, rANP stimulated [ $^3$ H] thymidine incorporation in a dose-dependent manner. Even at the lowest concentration ( $10^{-12}$  M) we examined, rANP showed a significant response.

### Discussion

Our findings demonstrated the novel effect of ANP on adrenal zona glomerulosa cells, i.e., growth stimulation. Because ANP could demonstrate this activity at concentrations as low as  $10^{-12}$  M, this strongly suggests that this growth stimulation is a physiological property of ANP, and that ANP is one of the hormones that regulates zona glomerulosa cell growth. It has been reported that ANP suppressed aldosterone production at zona glomerulosa cells, with a concomitant decrease in cAMP content and a concomitant increase in cGMP contents(7), and that cAMP worked as an inhibitor in terms of adrenal cell growth(8,9). In this context, it seems likely that ANP-induced changes in cyclic nucleotide are related to the

stimulation of zona glomerulosa cell growth, but the exact mechanism remains to be elucidated.

In summary, our findings demonstrated that, on cultured zona glomerulosa cells, ANP worked as a growth factor other than as a regulator for aldosterone production.

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