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Inability of Angiotensin to Stimulate RNA Synthesis in Isolated Rat Atria (38551)

RICHARD N. HILL, WALTER B. SEVERS, AND DAI KEE LIU
(Introduced by E. S. Vesell)

Department of Pharmacology, Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

The renin–angiotensin system has been implicated in the etiology of hypertension because of its potent vasoconstrictor properties and its effects on salt and water balance (1). Recent evidence suggested that this system may have other cardiovascular effects (2). Khairallah *et al.* (3) investigated the effect of angiotensin on the incorporation of radioactive precursors into DNA, RNA and protein of isolated rat atria in culture. They observed increased specific radioactivities of these macromolecules after not more than 2 hr exposure to the hormone. In addition, they reported that there was no stimulation of the uptake of nucleic acid precursors into the acid-soluble pool and that RNA content was increased in angiotensin treated tissues.

The present investigation was concerned with confirming these exciting observations associated with the stimulation of macromolecular synthesis by angiotensin. Concentrations of angiotensin spanning a 10,000-fold range were utilized, and several other variables were also investigated; but enhanced RNA synthesis in samples treated with angiotensin was not observed.

Materials and Methods. Atria were excised quickly from 150–200 g Sprague–Dawley rats sacrificed by decapitation. All animals were male, except where noted. The specific conditions employed were those reported by Khairallah *et al.* (3). Briefly, tissues were stored for not more than 30 min in ice cold Krebs–Hensleit solution containing 2 mg/ml glucose and equilibrated with 95% O_2–5% CO_2 to pH 7.4. They were then rinsed several times, blotted dry, and 4–6 atria were placed in Erlenmeyer flasks containing 10 ml of the above solution at 37° for 1 hr. The flasks were incubated in a rotating water bath and were bubbled constantly with the above gas mixture. Atria were transferred to flasks containing fresh salt solution; various concentrations of angiotensin and 1 μCi/ml [5-^{3}H]uridine (sp act 5 Ci/mM; Amersham/Searle) were added for 2 further hr of incubation. Incorporation into acid-precipitable material was linear for that period of time. Angiotensin II (a gift from CIBA) stock solutions (1 mg/ml) were made up in water each week, were stored in the refrigerator and were diluted just prior to use. Cultures were terminated by removing the tissues from the cultures and rinsing them several times in ice cold Hank's balanced salt solution. They were weighed and then homogenized (1:40 w/v) directly in ice cold 0.2 N perchloric acid (PCA). Duplicate samples were removed and were analyzed for acid-soluble and acid-precipitable, alkali-soluble fractions using the modified Schmidt–Thannhauser procedure recommended by Munro and Fleck (4). Specific activities of RNA were expressed as cpm/OD_{260}; the counting efficiency of our liquid scintillation counting system was 17%. RNA content was expressed as OD_{260}/g wet wt.

Results and Discussion. Doses of angiotensin between 1 ng/ml and 10 μg/ml were investigated. For the highest dose employed, some experiments were performed in which the saline was supplemented with a combination of amino acids which have been employed in heart perfusion studies (5). Every experiment included control cultures which did not contain angiotensin.

In analyzing the effects of angiotensin on the incorporation of [^{3}H]uridine into acid-insoluble material we used an analysis of variance design with treatment-control, dosage level, and study days as the main effects. Table I gives the mean values for each of the experimental-control dosage comparisons. The difference between the experimental and control grand means was tested by comparing the between-group variance to the within day-dosage-treatment error variance. There was a total of 45 observations, and the error

TABLE I. EFFECT OF ANGIOTENSIN ON THE INCORPORATION OF [^{3}H]URIDINE INTO RNA OF RAT ATRIA IN CULTURE.

Angiotensin concentration (ng/ml)	RNA specific activity (cpm/OD_{260})	
	Experimental	Control
1	1258.2[a]	1460.8[a]
5	1580.4	1305.1
10	1732.6	2043.6
50	2449.0	2554.5
1000	1196.0	1255.7
2000	509.0	537.6
10,000	2284.7	2084.3
10,000 + AA[b]	710.9	683.3
Grand mean	1465.1	1490.5

[a] All entries represent the mean of at least three replications of the experiment.

[b] Cultures supplemented with amino acids.

variance was 32,517.8. The F ratio was less than one and was clearly not significant. No significant interactions between experimental-control and dosage existed. Acid-soluble specific activities were comparable in treated and control incubations, and RNA contents were not altered in the angiotensin treated tissues.

Since Khairallah *et al.* (3) reported that 10 ng/ml of angiotensin was effective in increasing RNA synthesis, we investigated several other variables in experiments employing that dose of the hormone. (a) Preparation of the Krebs–Hensleit solution: The solution was prepared just before it was used or it was sterilized before use and/or penicillin and streptomycin were added during the experiments. (b) pH of the Krebs–Hensleit solution: By changing the concentration of bicarbonate in the gas-equilibrated solution, the initial pH of the medium was established at 7.15, 7.30, 7.40 and 7.60. (c) Medium composition: Earle's balanced salt solution was substituted for Krebs–Hensleit solution. (d) Weight of animals: Groups of male rats about 75, 150, 250 and 300 g were used. (e) Sex: Female rats weighing about 150 g or 250 g were employed. (f) Method of gassing with 95% O_2–5% CO_2 : Cultures were gassed by directing the mixture into a gas hood enclosing all of the cultures or into each of the culture flasks directly above the incubation medium. (g) Acid extraction: Tissues were extracted with cold 0.5 N PCA. No effect of angiotensin on the incorporation of [^{3}H]uridine into RNA was detected with any of these manipulations.

From the results of our experiments we conclude that angiotensin does not stimulate the synthesis of RNA under the conditions originally described (3). However, there are other independent reports claiming that angiotensin does have macromolecular effects: in cultured adrenal cells (6) it stimulates RNA synthesis but not protein synthesis, while in guinea pig atria (7, 8) it seems to augment protein synthesis as well as catecholamine synthesis. In both systems the conditions for eliciting the angiotensin-induced effects are stringent and depend upon several variables. At this time, further studies will be required to determine the experimental conditions for studying the effects of angiotensin on RNA synthesis in cultured rat atria.

Summary. Contrary to a previous report by other investigators, angiotensin was shown not to increase the incorporation of [^{3}H]uridine into RNA of rat atria in culture.

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