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LACK OF A DIRECT REGULATORY EFFECT OF ATRIAL NATRIURETIC FACTOR ON PROSTAGLANDINS AND RENIN RELEASE BY ISOLATED RAT GLOMERULI

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SUMMARY: We have tested the direct regulatory effect of synthetic Atrial Natriuretic Peptide (ANP, 8-33aa) on prostaglandins and renin release by isolated rat glomeruli. Variable incubation times and doses of ANP did not modify the rate of PGE_2, PGF_{2a} and TXB_2 production. Similar results were obtained for renin release. These data do not support a role for ANP in the regulation of prostaglandins and renin release by rat glomeruli.

There is now a lot of evidence that ANP-induced natriuresis is mainly mediated by hemodynamic changes (1,2), although some direct tubular effects can not be discarded (3,4). Moreover, the possibility that ANP controls directly intrinsic glomerular function can be hypothesized (5).

Some prostaglandins (PG), especially PGE_2 are powerful natriuretic autacoids and show vasodilator effects (6-8). It is conconceivable that PG could play a role in ANP-induced natriuresis. It has been reported that ANP infusion could modify urinary PGE_2 secretion (9). Thus, we decided to study the effect of ANP on PG release by isolated rat kidney glomeruli.

In addition, ANP has been reported to induce decrease in plasma renin activity (PRA) in some studies (2), but not in others (10) and it has not been defined whether this is a direct effect or it is mediated by the hemodynamic changes. Thus, we have measured the

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renin release by isolated rat glomeruli in presence or absence of ANP.

MATERIAL AND METHODS

Renal glomeruli were isolated from Wistar rats weighing 200-250 g according to a previously published technique (11,12). After ether anesthesia, a cannula was inserted into the abdominal aorta and isotonic cold heparinized saline (50-100 ml, 0.16M NaCl) was perfused. At the same time, aorta was clamped above renal arteries and vena cava was opened for drainage. Blanched kidneys were removed and immersed in ice-cold Tris-HCl buffer (Tris 20 mM, NaCl 130 mM, KCl 10 mM, Sodium acetate 10 mM, Glucose 5 mM, pH 7.45). All subsequent steps were performed with the same buffer.

Cortex from the kidney were dissected and minced to a paste-like consistency. The homogenate was pushed successively through a 106 µm-sieve which excluded the tubules and a 75 µm -sieve which retained glomeruli. The suspension obtained was centrifuged at 120 g for 3 min and the supernatant was discarded. This operation was repeated 3 times. The final pellet consisted of nearly pure isolated decapsulated glomeruli with a tubular contamination $< 3\%$.

Just before incubation, glomeruli were centrifuged (120 g, 3 min) and resuspended in a buffer similar to that used in the preparatory steps, with addition of 1 mM $CaCl_2$. Isolated glomeruli (500-800 µg of glomerular protein/tube) were incubated at 37ºC with continuous agitation (100 cycle/min) in a buffer volume of 1 ml, with or without ANP. ANP (8-33 aminoacids, Merck, Sharp & Dohme, 3.10^{-4}M) was added to the incubation tubes at time 0, in order to achieve final concentrations in the range 3.10^{-6}-3.10^{-12}. Incubation times were 10, 30 and 60 min. In a separate set of experiments, 5 µg/ml (final concentration) of arachidonic acid (AA, Sigma, St. Louis, Ma) was added to each incubation tube, with or without ANP. Incubations were stopped by centrifugation at 300 g for 3 min at 4ºC. Supernatants were collected and frozen until PG and renin radioimmunoassays were performed. Protein concentration was determined according to Lowry et al (13).

PG levels were measured directly in the protein-free supernatant of incubation media by RIA, without previous extraction (14, 15). (^3H)-PGE_2 (160 Ci.mmol^{-1}), (^3H)-$PGF_{2\alpha}$ (220 Ci.mmol^{-1}), (^3H)-TXB_2 (180 Ci.mmol^{-1}) and (^3H)-6-keto-$PGF_{1\alpha}$ (150 Ci.mmol^{-1}) were purchased from Amersham (U.K). Specific antibodies and PG standards were purchased from Seragen (Seragen Inc., Boston, MA). Intraassay and interassay variations were less than 4% and 16% respectively for all the PG tested. Renin concentration was determined by measuring the immunoassayable Ag I generated in excess of specific substrate (16).

Each point represents an average of 6-12 determinations. Results are expressed as $x \pm$ sem. Comparison was performed with one way and two ways analysis of variance. A $p < 0.05$ was considered statistically significant.

RESULTS

Table I shows the time-course of the PG synthesis by rat isolated glomeruli, in the presence or absence of ANP (3.10^{-9} M). The synthesis of the PGs studied increased with the time but an ANP fi-

TABLE I

Rates of PG synthesis ($ng \cdot mg^{-1}$ of protein) as a function of the time of incubation, with or without ANF ($3 \cdot 10^{-9}$)(C)

	10 min		30 min		60 min	
	C	ANF	C	ANF	C	ANF
PGE_2	7.2±0.3	7.4±0.7	7.5±0.2	7.7±0.3	8.8±0.5[a,b]	9.8±0.8[a,b]
$PGF_{2\alpha}$	2.9±0.5	2.9±0.1	3.5±0.2	3.5±0.1[a]	3.9±0.3	3.9±0.2[a,b]
6-keto-$PGF_{1\alpha}$	1.2±0.1	1.4±0.1	1.4±0.1[a]	1.6±0.1[a]	1.5±0.1[a]	1.6±0.1[a]
TxB_2	1.5±0.1	1.6±0.1	1.6±0.1	1.8±0.1	1.6±0.1	1.8±0.2

a: $p < 0.05$ vs 10 min.; b: $p < 0.05$ vs 30 min.

nal concentration of 3.10^{-9} did not modify this PG production rate.

Rat isolated glomeruli incubated for 30 min with different concentrations of ANP (Table II) did not show significant changes in PG release. When AA was added to the incubation media, the production of PGE_2 was clearly enhanced (twice the control value) in both control and ANP treated glomeruli (Fig. 1).

Significant amounts of renin, detectable in the incubation media by RIA, were produced by isolated glomeruli incubated for 30 min in basal conditions. Variable doses of ANP did not influence this renin release rate (Table III).

TABLE II

Dose-response of PG production ($ng \cdot mg^{-1}$ in presence of ANF)

ANF (M)	PGE_2	$PGF_{2\alpha}$	6-keto-$PGF_{1\alpha}$	TxB_2
0	7.6±0.2	3.5±0.2	1.3±0.1	2.2±0.1
$3 \cdot 10^{-12}$	7.4±0.3	3.6±0.4	1.3±0.1	2.1±0.1
$3 \cdot 10^{-11}$	7.2±0.4	3.7±0.3	1.2±0.1	2.3±0.2
$3 \cdot 10^{-10}$	7.3±0.7	3.9±0.2	1.3±0.1	2.1±0.1
$3 \cdot 10^{-9}$	7.8±0.3	3.1±0.2	1.2±0.1	2.1±0.1
$3 \cdot 10^{-8}$	7.1±0.6	3.2±0.4	1.4±0.1	2.2±0.1
$3 \cdot 10^{-7}$	6.8±0.7	3.5±0.4	1.2±0.1	2.2±0.2
$3 \cdot 10^{-6}$	6.5±0.8	3.3±0.4	1.3±0.2	2.0±0.1

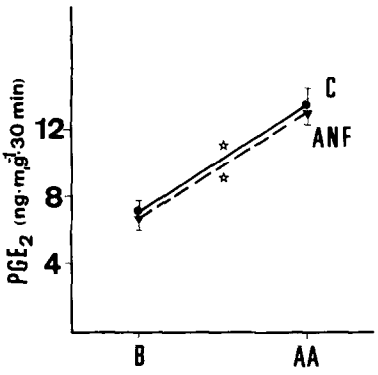

Figure 1. Basal (B) and archidonic acid stimulated (AA) glomerular production of PGE_2. C: control glomeruli incubated with atrial natriuretic factor ($3.10^{-9}M$).
*$p < 0.05$ vs B.

DISCUSSION

The production of PG by isolated glomeruli has been demonstrated by previous reports (11,12,14,15). This release can be stimulated by AA and vasoactive hormones (14,15). In our study, PGE_2 is quantitatively the most important AA cyclo-oxygenase metabolite produced by the glomeruli, but significant amounts of $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 are also detected. ANP did not modify the basal and AA-stimulated rate of PG release at various concentrations and incubation times. This result suggests that ANP action is independent of PG production by the glomeruli. These data are in agreement with those of Keeler, who demonstrated that ANP effect on kidney persisted after PG inhibition (17).

TABLE III

Renin Release (RR, $\mu U \cdot mg^{-1}$ protein/30 min) by isolated glomeruli incubated with different Doses of ANF

ANF (mol/l^{-1})	0	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}
RR	6.5±1.1	6.3±1.0	6.1±1.3	7.0±0.7	5.8±1.7	6.9±1.0

However, these results must be viewed with caution. Glomeruli are heterogenous structures, with three main types of cellular species which synthetize PG at different rates (11,18,19). ANP stimulating or inhibiting effect on one specific cellular type may be masked when the complete glomeruli are studied.

Recently, Ishii and coworkers (9) have found a significantly positive correlation between urinary sodium and PGE_2 excretion after infusion of an atrial extract. They have suggested a contribution of this PG to the natriuretic effect of atrial extract. It seems possible that tubular or interstitial structures account for this increase in PGE_2 production. Moreover, this PGE_2 increase could be independent of ANP and dependent on the changes in urinary flow (20) induced by ANP (5).

Study of renin production by isolated glomeruli (21) offers the advantage of the renal subunit independence of external influences (22), with maintenance of the local anatomical structure. Different doses of ANP did not modify the rate of renin release by isolated glomeruli. However, Burnett and coworkers (2) have described an inhibition of renin production by ANP infusion in anaesthetized dogs whereas Hackental and coworkers (10) have reported the opposite effect in isolated rat kidney. Considering our and previous results, it is possible that ANP does not induce changes in the rate of renin production by a direct action but through indirect influences in the complex system of renin regulation (22).

In conclusion, these results demonstrate that ANP does not modify PG and renin release by isolated rat glomeruli.

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