Rat Atrial Natriuretic Peptides Inhibit Oxygen Consumption by Rat Kidney

NORIYUKI KOHASHI, NICK C. TRIPPODO, ALLAN A. MACPHEE, EDWARD D. FROHLICH, AND FRANCIS E. COLE

SUMMARY The inhibitory effect of high and low molecular weight native and synthetic rat atrial peptides on oxygen consumption in isolated rat kidney mitochondria and slices was measured. Oxygen consumption by mitochondria was measured in the presence of succinate and/or adenosine diphosphate, furosemide, and low and high molecular weight native and synthetic rat atrial peptides. After the addition of succinate, adenosine diphosphate limiting respiration (State 4) increased in the presence of low, but not high, molecular weight native rat atrial peptides. Furosemide caused a significant decrease in State 4 respiration (p < 0.001). Angiotensin II and arginine vasopressin did not alter State 4 respiration. The rate of oxygen consumption after the addition of saturating adenosine diphosphate in the presence of saturating succinate (State 3 respiration) was reduced by low and high molecular weight native rat atrial peptides. Furosemide completely blocked oxygen consumption after the addition of adenosine diphosphate. Oxygen consumption was unchanged by trypsin treated (natriuretically inactive) low molecular weight rat atrial peptides and ventricular protein extracts of high and low molecular weight native rat atrial peptides. Synthetic and low molecular weight native rat atrial peptides had similar effects on mitochondrial oxygen consumption. Low molecular weight native and synthetic rat atrial peptides decreased the adenosine diphosphate to oxygen ratio, and these peptides, as well as furosemide, also induced mitochondrial swelling; none of the other rat atrial peptide combinations nor angiotensin II produced this effect. In kidney slices, basal oxygen consumption (without substrates) was stimulated by succinate. This succinate-stimulated oxygen consumption was inhibited when kidney slices were incubated with synthetic rat atrial peptides. Synthetic and native atrial peptides had indistinguishable inhibitory effects on renal oxygen consumption. Native rat atrial peptides and furosemide inhibited renal oxygen consumption and induced mitochondrial swelling. These events may be related to their rapid natriuretic effect.

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KEY WORDS • natriuresis • renal • oxygen consumption • mitochondria • atrial natriuretic peptides

CARDIAC atrial myocytes contain potent, fast-acting natriuretic peptides observed in the rat by de Bold et al. in 1981.1 This family of natriuretic peptides has been demonstrated in the atria of a number of mammals, including humans.2-4 Recently, several forms of rat (rAP) and human atrial peptides were sequenced, and though they differ in primary structure, each of the C-terminal peptides exhibits potent natriuretic and diuretic activity.7-17 Their natriuretic effect appears to result from inhibition of sodium reabsorption in renal medullary collecting tubules and ducts,18,19 but decreased renal vascular resistance also might be involved.10,20

The mechanism by which rAP induce natriuresis is unknown. Because concentrating sodium against a concentration gradient is an energy-requiring process,21 a decrease in oxidative cellular metabolism in renal cells responsible for sodium retention would be expected to favor natriuresis. The site of oxidative energy production is the mitochondria. Specific metabolic inhibition of renal cells by the natriuretic drug furosemide (FU) was reported by Mohr,22 who observed submicroscopic alterations of tubular epithelial mitochondria during peak diuresis after a single dose of FU. After diuresis, mitochondria were regenerated and enzyme activities returned to normal levels. To

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investigate a potential relationship between oxidative metabolism and natriuresis and diuresis for rAP, we compared the effects of FU with those of synthetic and native rAP on oxygen consumption (O$_2$C) by kidney mitochondria and slices.

**Methods**

**Chemicals, Drugs, and Atrial Peptides**

Angiotensin II (ANG II), aprotinin, and bovine serum albumin were purchased from Sigma Chemical Corporation, St. Louis, MO, USA. Arginine vasopressin (AVP, Pitressin) was purchased from Parke-Davis, Morris Plains, NJ, USA, and FU was purchased from Elkins-Sinn, Inc., Cherry Hill, NJ, USA. Synthetic rat atriopeptin III (rAP III) was purchased from Peninsula Laboratories, Inc., San Carlos, CA, USA.

Partially purified native rAP were prepared as described previously.$^{4,5}$ Briefly, atria were obtained from 200 male Sprague-Dawley rats, homogenized in 1 M acetic acid (10 ml/g of tissue), and centrifuged at 1200 × g for 10 minutes at 4°C. The pellet was homogenized, reextracted in one half the volume of acetic acid, and recentrifuged. The supernatants were combined and lyophilized. The lyophilized extract was homogenized in 1 M acetic acid and centrifuged, and the supernatant was fractionated on Sephadex G-75 eluted with 1 M acetic acid. High molecular weight rAP (HMW) were obtained from the 10,000- to 30,000-dalton fraction, and low molecular weight rAP (LMW) from the 3,000- to 10,000-dalton fraction. These fractions were lyophilized and dissolved in Krebs-Ringer bicarbonate buffer (KRBB) for bioassay of natriuretic activity and were tested for their effects on oxygen consumption by kidney slices and isolated kidney mitochondria. For HMW, 5.1 mg of protein was dissolved in 1.0 ml of KRBB and 0.1 ml added to incubation vials containing 3 ml final volume. For LMW, 3.0 mg of protein was dissolved in 3.8 ml of KRBB and 0.1 ml was also used. These amounts of HMW and LMW produced near maximum natriuresis (>400 µEq/kg/10 min) in anesthetized assay rats$^4$ and had approximately equivalent natriuretic activity. In 11 assay rats weighing 175 ± 12 g (±SD), 210 µg of protein of HMW elicited a change in urinary sodium excretion of 145 ± 107 µEq/kg/10 minutes from a control of 19 ± 17. By comparison, in 14 assay rats weighing 189 ± 11 g, 38 µg of protein of LMW increased urinary sodium excretion by 182 ± 119 µEq/kg/10 minutes from control of 14 ± 10. A 2-µg infusion of rAP III in 1.0 ml of phosphate buffered saline (8 × 10$^{-7}$ M) produced a similar natriuresis in bioassay rats. Inactive (natriuretically) atrial extract was obtained by treating LMW with a suspension of bovine pancreatic trypsin coupled to agarose (Sigma) as previously described.$^5$

High (HMW) and low (LMW) molecular weight ventricular extracts were prepared according to the same procedures used for atrial extracts. The doses of HMW and LMW studied contained approximately the same amount of protein used for studies of their respective atrial extracts.

**Preparation of Rat Kidney Mitochondria and Tissue Slices**

Adult male Sprague-Dawley rats (360 ± 24 g) were killed by decapitation. Rat kidney mitochondria were prepared by a modification of the method of Weinbach.$^2$ Kidneys were rapidly removed and transferred to ice-cold 0.25 M sucrose. The kidneys were crushed in an ice-chilled cylindrical tissue press (Harvard Apparatus Co., Inc., Millis, MA, USA). Each gram of crushed tissue was added to 9 ml of ice-cold 0.25 M sucrose and homogenized for 2 minutes with a Teflon glass Potter-Elvehjem type homogenizer (Thomas Co., Philadelphia, PA, USA). The homogenates were centrifuged at 600 g for 10 minutes at 4°C. The supernatant was transferred to prechilled plastic tubes and centrifuged at 8500 g for 10 minutes. The crude mitochondrial pellet was washed three times with 2 ml of 0.25 M sucrose per gram of kidney tissue and finally suspended in 0.25 M sucrose (1 milligram per gram of tissue). The O$_2$C of the mitochondria was measured within 1 hour of preparation.

Kidney slices were prepared from KRBB-perfused rat kidneys. After induction of pentobarbital anesthesia (50 mg/kg), a polyethylene catheter (PE-90) was inserted into the abdominal aorta immediately below the renal arteries. Subsequently, the aorta was clamped above the renal arteries, the left renal vein cut, and the proximal end of the aorta connected by the catheter to a 50-ml syringe in the infusion pump (Harvard Apparatus, Millis, MA, USA). Cold KRBB perfusion medium was infused at 7.64 ml/minute. Perfusion was continued until both kidneys were blanched. The kidneys were removed and immediately placed in ice-cold 0.9% saline. Kidneys were sliced sagittally with a Stadie-Riggs microtome (Thomas Co., Philadelphia, PA, USA), and the slices were placed in ice-chilled KRBB. Only center slices (125–175 mg wet weight) were used.

**Oxygen Consumption**

The O$_2$C was measured polarographically with the method of Chance and Williams$^4$ with a Y.S.I. Model 53 biological oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, OH, USA). For mitochondrial O$_2$C, each incubation flask contained a final volume of 3 ml consisting of 75 mM KCl, 50 mM Tris-hydrochloride, 12.5 mM K$_2$HPO$_4$, 5 mM MgCl$_2$, and 1 mM EDTA, pH 7.4 at 25°C. This medium was saturated with 95% O$_2$, 5% CO$_2$ for 5 minutes before the addition of mitochondria, substrates, or substance being tested for the effect on O$_2$C. A typical incubation mixture contained 2.4 ml of incubation buffer, 0.3 ml of mitochondria suspension, 0.1 ml of adenine diphosphate (ADP, 250 µM final concentration), 0.1 ml of succinate (7 mM final concentration), and 0.1 ml of test substance (e.g., rAP, ANG II). Control incubations contained 2.5 ml of incubation buffer and no test...
substance. The oxygen concentration before adding the mitochondrial suspension was calculated to be 250 μM. The slopes of the polarographic tracing (m1, m2, and m3) were used to calculate the rate of O2C and were expressed as nanoliters of O2 per milligram of protein per minute (see Figure 1). By definition, during State 4 respiration O2C is limited by ADP only, whereas during State 3 respiration O2C represents the capacity of the respiratory chain in the presence of saturating substrates.

Respiratory Control Index

The RCI was calculated from the ratio of State 3 to State 4 respiration. The RCI is an index of the tightness of coupling of O2C and phosphorylation in mitochondria. Thus, RCI = State 3 (O2C nl/min/mg of protein)/State 4 (O2C nl/min/mg of protein), or as illustrated in Figure 1 (m1/m2). The RCI values using succinate as a substrate range from 3 to 5.

Adenosine Diphosphate to Oxygen Ratio

The ADP to oxygen (ADP:O) ratio was calculated according to Estabrook as follows. The distance Y (see Figure 1) is determined by extending the slope lines m1, m2, and m3 and measuring the number of recorder divisions from the intersection of lines m1-m2 and m2-m3. The number of divisions is proportional to the ADP-stimulated respiration (State 3). The recorder deflection X represents the total oxygen content of the reaction mixture, which was estimated to be 250 μM. Microatoms of oxygen used = (Oxygen content of medium/X units) × Y units × 3 ml of reaction medium × 2. The ADP added per microatom of oxygen used = ADP:O ratio. The ADP:O ratio indicates the ability of mitochondria to conserve the energy produced by substrate oxidation in a form that can be used in the cell. As one of the phosphorylation sites is bypassed with coupled succinate oxidation, the maximum ADP:O ratio attainable is 2.0.

In the present study, the mean range of RCI in control incubations was from 3.3 to 4.1 and the mean range of ADP:O ratio was from 1.76 to 2.00, both characteristic of well-preserved mitochondrial function.

To measure O2C in kidney slices, KRBB saturated with 95% O2, 5% CO2 for 3 minutes. The RCI values using succinate as a substrate range from 3 to 5.

Mitochondrial Swelling

Mitochondrial swelling was measured by following the change in absorbancy of the mitochondrial suspension at 520 nm in a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH, USA) with a modification of the method of Lehninger and Neubert. Swelling experiments were performed in 15-× 85-mm disposable culture tubes (American Hospital Supply Corporation, McGaw Park, IL, USA). The reaction was started by diluting 0.05 ml of the stock suspension of mitochondria (0.5 mg of mitochondrial protein) to 5.0 ml of 0.125 M KC1, 0.02 M Tris-hydrochloride medium at pH 7.3 that already contained the other additions (substrates to be tested for their effect on swelling), previously adjusted to this pH at 25°C. The data are expressed in terms of the decline in absorbancy × 102 of mitochondria at 520 nm (i.e., Δ log Io/I × 102 at 520 nm) produced by the swelling agent above the spontaneous swelling rate at 30 minutes. Protein concentrations in all experiments were determined by the method of Lowry et al.

Statistics

Statistical comparisons between control and treated groups were analyzed by one-way (Tables 1–3) and two-way (Figure 2) analyses of variance with subsequent Neuman-Keuls tests where necessary. Data are presented as mean ± SD (Tables 1–3) or mean ± SEM (Figure 2).

Results

Renal Mitochondrial Oxygen Consumption

Typical polarographic recordings of the O2C by isolated kidney mitochondria in the presence of substrates and test substances are shown in Figure 1. In each set of experiments, control incubations (without test substance) were compared with separate incubations containing test substances (e.g., HMW, LMW, FU, etc.) using the same mitochondrial preparation. Sets were repeated with separate mitochondrial preparations. Doses of LMW (79 μg protein) and HMW (210 μg protein) produced equivalent, near maximum natriuresis bioassay rats. Preparations of LMWV, HMWV, and trypsin-treated LMW (LMWT) were tested for their effects on O2C using amounts of protein comparable to their natriuretically active counterparts. Basal O2C, with no substrates added, was minimal. The addition of saturating amounts of succinate and ADP increased O2C in an additive manner.

State 4 respiration was unchanged after the addition of HMW but increased after LMW (Table 1). State 3 respiration was reduced in the presence of HMW and more so in the presence of LMW. The RCI remained unchanged after the addition of HMW but decreased when mitochondria were exposed to LMW. The mitochondrial ADP:O ratio decreased after exposure to LMW but was unchanged when exposed to HMW. In addition to completely blocking the O2C after adding ADP, FU administration also significantly decreased State 4 respiration (p < 0.001). There were no signifi-
Figure 1. Polarographic recording of O2C of mitochondria isolated from normal rat kidneys. (A) O2C incubated without additives. (B) O2C incubated with LMW. (C) O2C incubated with HMW. (D) O2C incubated with FU (4 mM). Incubations were in a final volume of 3 ml, as described in the text. Calibration of the oxygen electrode was based on the calculated value of initial oxygen content of water saturated with room air at 25°C.24 I = O2C of 95% O2, 5% CO2 saturated medium; II = O2C after addition of 0.3 ml of mitochondria, with or without rAP or FU; III = O2C after addition of 7 mM succinate; IV = O2C after addition of 250 μM ADP; m = slope (-----); X and Y are described in Methods.

Table 1. Effect of Low and High Molecular Weight Rat Atrial Peptides, Similarly Prepared Low Molecular Weight Extract from Rat Ventricles, a Similar Preparated High Molecular Weight Extract from Paired Rat Ventricles, Low Molecular Weight Rat Atrial Peptides Treated with Trypsin, Furosemide, Angiotensin II, and Arginine Vasopressin on the Rate of Rat Kidney Mitochondrial Oxygen Consumption in the Presence of Saturating Substrate (7 mM succinate, State 4 respiration, and 250 μM adenosine diphosphate + 7 mM succinate, State 3 respiration). O2C was expressed as nl O2/min/mg protein.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Respiration</th>
<th>RCI</th>
<th>ADP:O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>State 4 (nl of O2/min/mg)</td>
<td>State 3 (nl of O2/min/mg)</td>
<td></td>
</tr>
<tr>
<td>Control (19)</td>
<td>218 ± 26</td>
<td>829 ± 92</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>LMW (17)</td>
<td>275 ± 42</td>
<td>645 ± 69*</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>HMW (14)</td>
<td>218 ± 28</td>
<td>761 ± 100*</td>
<td>3.5 ± 0.7</td>
</tr>
<tr>
<td>Control (11)</td>
<td>266 ± 31</td>
<td>871 ± 100</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>LMWV (10)</td>
<td>266 ± 47</td>
<td>863 ± 87</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Control (10)</td>
<td>238 ± 37</td>
<td>948 ± 130</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>HMWV (10)</td>
<td>257 ± 51</td>
<td>909 ± 60</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>Control (10)</td>
<td>170 ± 27</td>
<td>656 ± 68</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>LMWT (10)</td>
<td>161 ± 18</td>
<td>634 ± 54</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>Control (10)</td>
<td>236 ± 23</td>
<td>869 ± 91</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>FU 4 x 10^-7M (12)</td>
<td>159 ± 242</td>
<td>159 ± 242</td>
<td>1.0 ± 0</td>
</tr>
<tr>
<td>Control (11)</td>
<td>202 ± 35</td>
<td>779 ± 60</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>ANG II 10^-6M (11)</td>
<td>204 ± 32</td>
<td>691 ± 48*</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>Control (8)</td>
<td>210 ± 24</td>
<td>861 ± 97</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>AVP 10^-7M (8)</td>
<td>204 ± 20</td>
<td>725 ± 67*</td>
<td>3.6 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± sd.
The number of repetitions is indicated in parentheses.
AVP = arginine vasopressin; ANG II = angiotensin II; ADP = adenosine diphosphate; FU = furosemide, HMW = high molecular weight rat atrial natriuretic peptides (rAP), HMWV = high molecular weight extract from rat ventricles; LMW = low molecular weight rAP; LMWV = low molecular weight extract from paired rat ventricles; LMWT = low molecular weight rAP treated with trypsin; RCI = respiratory control index; ADP:O = ADP to oxygen ratio.

*p < 0.05, **p < 0.01, ***p < 0.001, using one-way analysis of variance between control and treatment groups.
RAT ATRIAL PEPTIDES INHIBIT KIDNEY OXYGEN CONSUMPTION

Kohashi et al.

495

cant differences in mitochondrial O$_2$C as a result of exposure to LMW treated with trypsin compared with control. Similarly, LMWT did not induce natriuresis in bioassay rats. The addition of ANG II (10$^{-6}$ M) decreased State 3 respiration and decreased the RCI. Lower concentrations of ANG II (10$^{-8}$ and 10$^{-9}$) did not affect O$_2$C (data not shown). Like ANG II, AVP also decreased State 3 respiration and the RCI. These AVP effects were observed at concentrations as low as 10$^{-9}$ M.

Natriuretic doses of synthetic rAPIII were tested in this system. As seen in Table 2, the O$_2$C after the addition of saturating succinate increased after 5.2 × 10$^{-7}$ M rAPIII was added. State 3 respiration was reduced by 2.6 × 10$^{-7}$ M rAPIII. The RCI also decreased in the presence of rAPIII, and the mitochondrial ADP:O ratio decreased after exposure to rAPIII.

**Swelling of Mitochondria**

As shown in Table 3, rAPIII, LMW, and FU caused a profound mitochondrial swelling within 30 minutes, whereas HMW, LMWV, LMWT, and ANG II did not. This effect was rapid, being detectable between 5 and 10 minutes after the swelling agent was added (data not shown).

**Oxygen Consumption of Kidney Slices**

Whereas LMW clearly affected O$_2$C at the subcellular level, initially we were unable to demonstrate an effect of LMW or HMW on O$_2$C in rat kidney slices. As native rAP did not change the O$_2$C in kidney slices during a 3-minute observation, we investigated the effects of a longer period of exposure by preincubating slices with rAPIII under conditions designed to minimize proteolysis, as shown in Figure 2. While basal

<table>
<thead>
<tr>
<th>Table 2. Effect of Synthetic Atriopeptin III on Mitochondrial Oxygen Consumption in the Presence of 7 mM Succinate and 250 μM Adenosine Diphosphate.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental condition</strong></td>
</tr>
<tr>
<td>------------------------------</td>
</tr>
<tr>
<td>Control (7)</td>
</tr>
<tr>
<td>rAPIII* (2.6 × 10$^{-7}$ M) (7)</td>
</tr>
<tr>
<td>Control (6)</td>
</tr>
<tr>
<td>rAPIII* (5.2 × 10$^{-7}$ M) (6)</td>
</tr>
</tbody>
</table>

Values are means ± SD. The number of repetitions is indicated in parentheses.

ADP = adenosine diphosphate; ADP : O = ratio of adenosine diphosphate to oxygen; rAPIII = synthetic rat atriopeptin III; RCI = respiratory control index; State 4 = 7 mM succinate; State 3 = 250 μM ADP + 7 mM succinate.

*Synthetic rat APIII produced maximum natriuresis in bioassay rats (Lot No. 005642, MW 2550).

$\ast p < 0.001$, using one-way analysis of variance between control and treatment groups.

<table>
<thead>
<tr>
<th>Table 3. Effect of Test Substances on Swelling of Rat Kidney Mitochondria</th>
</tr>
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<tbody>
<tr>
<td><strong>Test substance</strong></td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Rat atriopeptin III (8)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Native rat atrial peptide</td>
</tr>
<tr>
<td>LMW (6)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>HMW (7)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>LMWV (8)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>LMWT (4)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Furosemide (6)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Angiotensin II (5)</td>
</tr>
</tbody>
</table>

Values are means ± SD. The number of repetitions is indicated in parentheses.

HMW = high molecular weight; LMW = low molecular weight; LMWT = trypsin treated LMW rat atrial peptides; LMWV = LMW ventricular protein extracts; OD = optical density.

*These doses of atrial peptide cause near equivalent maximum natriuresis in bioassay rats.

$\ast p < 0.001$, using one-way analysis of variance between control and treatment groups.
O₂C was inhibited by 3 mM FU (data not shown), it was unchanged by rAP III. This basal rate was stimulated in a dose-dependent manner in the presence of 7 mM succinate and 14 mM succinate. This stimulation was inhibited by rAPIII (260 nM) in 7 mM succinate or in 14 mM succinate.

### Discussion

In the present study, we found that rAP inhibited succinate-stimulated O₂C in kidney slices and inhibited State 3 respiration and decreased the ADP:O ratio in isolated kidney mitochondria. Synthetic rAP III showed effects on renal O₂C indistinguishable from those of native LMW.

We found that atrial peptides, ANG II, and AVP inhibited mitochondrial State 3 respiration and reduced RCI. Infusions of synthetic atrial peptides produced detectable natriuresis at doses similar to those concentrations needed to inhibit renal O₂C (i.e., 10⁻⁷ M). Snart demonstrated a comparable potency for AVP when he observed that it produced maximal sodium transport in mitochondria at 10⁻⁹ M. In contrast, the dose of ANG II required to stimulate aldosterone synthesis appears to be much lower than that needed to produce the mitochondrial O₂C inhibiting effects that we observed. As the circulating level of ANG II is 10⁻⁶ M and AVP is 10⁻¹² M, the effects on mitochondrial O₂C may not have been physiological, but hormone concentrations at intracellular mitochondrial sites could be higher. Although physiological levels of rAP have not been reported, the 10⁻³ M rAP III necessary to inhibit mitochondrial O₂C also seems high.

From these experiments, it is not possible to decide whether the inhibitory effects we observed occur in renal vascular or renal tubular elements, and the sensitivity of the response may increase when preparations enriched in tubular or vascular cells are used. Interestingly, our laboratory recently observed that ANG II and AVP, but not norepinephrine, greatly potentiated the natriuretic activity of LMW.

The importance of structural differences in the atrial natriuretic peptides and their mechanisms of action in promoting renal sodium excretion have not been defined. An intact disulfide ring has been demonstrated to be essential for natriuretic activity. The C-terminal disulfide ring portion of human atrial peptides differs in one amino acid from that of rAP, but it has natriuretic activity in the rat. The importance of chain length also has not been established. Peptides of various lengths have been isolated from the same species, and Forssmann et al. have reported that part of the N-terminal sequence of the probable precursor peptide has vasorelaxant but not natriuretic activity. Currie et al. reported that, in contrast to LMW, the HMW were relatively inactive when tested on smooth muscle preparations in vitro. Our finding that LMW were more active than HMW in affecting renal O₂C at the mitochondrial level in vitro is consistent with these data. Several investigators have suggested a precursor-product relationship between HMW and LMW. Further, we have observed that HMW was converted to LMW by atrial extracts and platelets. Thus, to be active, HMW may require conversion to LMW, either during its release or in blood. The low activity of HMW in isolated mitochondria (shown in Table 1) may therefore represent a lack of sufficient conversion.

We compared the effects of FU and rAP on renal O₂C because both rapidly induce a short natriuresis. As far as we know, their effects on renal O₂C have not been compared in the same preparation previously. The rapid natriuretic effect of FU is more prolonged than that of rAP in rats. To compare their effect on O₂C (see Tables 1 and 2), we chose doses of FU, rAP, and rAP III that produced near maximum natriuresis in bioassay rats. We confirmed the observation that FU (3 mM) inhibited O₂C in kidney slices, though less potently than that reported by other investigators, who observed O₂C inhibition by 5.2 x 10⁻⁴ M FU in potassium-free incubation media. Further, 4 mM FU inhibited State 4 respiration but completely blocked O₂C following ADP addition in isolated mitochondria. In contrast, 260 nM rAP III-inhibited succinate stimulated O₂C in kidney slices while 260 nM rAP III increased State 4 respiration in mitochondria. Like FU, rAP III inhibited State 3 respiration, but not completely. Thus, at doses needed to produce near equivalent maximum natriuresis, FU on a molar basis appears to be much less potent than rAP in inhibiting O₂C. These data demonstrate differences between FU and rAP in potency and mechanism in inhibiting renal O₂C.

As to the mechanism of action of these diuretics, the chloride-dependent Na-K cotransport system in erythrocytes in the presence of ouabain has been suggested...
as a model that can be used to study loop diuretics. Further, whereas FU inhibits Na\(^+\)-K\(^+\)-activated ATPase, rAP does not inhibit Na-K dependent ATPase from pork cerebral cortex, dog kidney, or rat renal medulla.

Our results provide evidence that FU and rAP inhibit mitochondrial O\(_2\)/C by clearly different mechanisms and induce mitochondrial swelling. Swelling of mitochondria, inhibition of O\(_2\)/C, and oxidative phosphorylation are related events. Thus, FU and rAP through these actions, if they occur in vivo, would contribute metabolically to an adenosine triphosphate deficient state, a condition supportive of their observed natriuretic effects.

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References

9. Flynn TG, de Bold ML, de Bold AJ. The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. Biochem Biophys Res Commun 1983; 117:859–865
39. Herrms W, Kersting F. Studies on oxygen consumption and change of tissue cation concentration in K\(^+\) depleted rabbit kidney slices, under the influence of diuretic agents, p-chloromercuribenzoate and N-ethylmaleimide. In: Rohr-Ramen F,

Ellory JC, Stewart GW. The human erythrocyte Cl-dependent Na-K cotransport system as a possible model for studying the action of loop diuretics. Br J Pharmacol 1982;75:183-188


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