Natriuretic Activity of Human and Monkey Atria

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SUMMARY. Recent evidence indicates that mammalian atria contain a substance that produces a rapid onset natriuresis in anesthetized rats. In the present experiments, small portions of human right atria obtained from patients undergoing coronary bypass surgery, as well as monkey atria and ventricles obtained from the Regional Primate Center, Seattle, were boiled or acid extracted and lyophilized. These materials (30 mg/kg) were dissolved in Ringer's lactate and injected intravenously into anesthetized monkeys to determine their effects on renal function. Their effects were also compared with those of furosemide (0.1 mg/kg) and chlorothiazide (10 mg/kg). Human and monkey atrial extracts produced significant increases in sodium and calcium excretion that were independent of changes in creatinine clearance. Monkey ventricular extract had no consistent renal effects. Furosemide, but not chlorothiazide, mimicked the renal responses to human and monkey atrial extracts in terms of time of onset, duration, and pattern of electrolyte excretion. These data suggest that primate atrial tissue contains a heat- and acid-stable natriuretic factor similar to that first described in the rat, suggesting that mammalian atrial natriuretic factors are cross-reactive among species. In addition, atrial natriuretic factors may have a mechanism of action similar to that of furosemide. (Circ Res 53:420-423, 1983)

ATRIAL MYOCYTES of the mammalian heart contain membrane-bound granules similar to those found in polypeptide-hormone-producing cells (de Bold et al., 1978). These granules were first reported by Kish in 1956. Sodium deprivation leads to atrial hypergranulation, whereas sodium loading causes degranulation (de Bold, 1979). Intravenous injection of extracts of rat or rabbit atrial, but not ventricular, myocardium causes an immediate natriuresis and diuresis that is independent of the central nervous system (de Bold et al., 1981; Trippodo et al., 1982, 1983). It has also been shown that the atrial extract acts directly on the kidney by mechanisms which do not depend on prostaglandin synthesis (Keeler, 1982), and it has been suggested that the natriuresis is due mainly to inhibition of sodium reabsorption in the medullary collecting duct (Sonnenberg and Cupples, 1981; Briggs et al., 1982).

The purposes of the present experiments were to determine whether a natriuretic factor could be extracted from human and monkey atria, and whether they are active in the primate kidney, and if so, to compare the pattern of renal responses of this substance with two diuretics which are believed to act at different renal sites.

Methods

Small portions of human right atrial appendages (HA) were obtained from patients undergoing coronary bypass surgery. Approval was obtained from the University's Institutional Review Board for the protection of human subjects. The tissue was immersed in ice cold, isotonic saline and then frozen at −70°C. Frozen monkey atria (MA) and ventricles (MV) were obtained from the Regional Primate Research Center at the University of Washington, Seattle. The tissues were either boiled (Trippodo et al., 1982) or acid-extracted (de Bold, 1982) and stored at −70°C until used.

Boiled Extraction

The tissue was homogenized in a blender in 5–10 parts of phosphate-buffered saline (0.140 M NaCl, 0.003 M NaH2PO4, 0.007 M Na2HPO4) per gram of wet tissue, transferred to a hot plate, and boiled for 10 minutes. The homogenate was centrifuged at 11,700 g for 10 minutes and the supernatant was filtered through gauze, lyophilized, and stored in a freezer.

Acid Extraction

The tissue was homogenized in a blender in 10 parts of 1.0 N acetic acid per gram of wet tissue, allowed to stand at room temperature for 15 minutes, and then centrifuged at 11,700 g for 10 minutes. The supernatant was saved and its pH was adjusted to approximately 8.2 with concentrated NH4OH. The solution then was centrifuged at 11,700 g for 10 minutes, and the supernatant was filtered through gauze, lyophilized, and stored in a freezer.

The experiments were performed on female Macaca fascicularis monkeys weighing 2.4–3.4 kg, maintained on normal monkey chow, and allowed water ad libitum. The animals were sedated with ketamine-HCl administered intramuscularly, followed by 30 mg/kg of pentobarbital sodium administered intravenously, and supplemented as needed to maintain a uniform level of anesthesia.

A small incision was made in the femoral area, and the femoral vein was cannulated for fluid administration and blood withdrawal. In addition, an angiocath (Deseret Company) was introduced into the ipsilateral femoral artery and connected to a transducer (Millar Instruments) for measurement of arterial blood pressure. A bladder catheter was inserted through the urethra to obtain urine...
at 10-minute intervals, at which time the bladder was gently compressed until air cleared the catheter. Ringer’s lactate solution was infused into the femoral vein at a rate of 4 ml/kg per hour.

After four 10-minute periods, during which urine flow did not vary by more than 10%, the dry extract of human or monkey atrial tissue (30 mg/kg) was dissolved in 5 ml Ringer’s lactate solution and injected into each of five monkeys intravenously over a 1-minute period. An injection of MV (30 mg/kg), furosemide (FU) (0.1 mg/kg), or chlorothiazide (CT) (10 mg/kg) was then given at least 2 hours after the atrial injection. Blood samples were taken 5 minutes before and 15 minutes after each injection and at the end of the experiment. All blood samples were replaced with an equal volume of 6% dextran in isotonic saline. At the completion of the experiment, the catheters were withdrawn, the incisions sutured, and the animals returned to their cages to recover from the anesthetic. No post-experimental complications occurred.

Endogenous creatinine clearance (Ccr) was used as an index of GFR (Levinsky and Levy, 1973). Although there appears to be some tubular secretion of creatinine in the primate, its clearance is a useful index of changes in glomerular filtration rate (GFR). Plasma and urine creatinine were determined with an autoanalyzer, Na+ and K+ were determined by flame photometry, Ca ++ by atomic absorption spectrophotometry, and osmolality by freezing point depression. Clearances were calculated in the standard manner.

### Statistical Analysis

Since there was no difference in the response to the acid or boiled extracts, the data were pooled for statistical analysis. The significance of differences among sample means was assessed by a one-way analysis of variance and Duncan’s new multiple range test. Differences are described as statistically significant if P < 0.05.

### Results

The mean renal responses of the monkey to the injection of monkey and human atrial extract are shown in Tables 1 and 2, respectively. In response to the atrial extract, a renal response was observed within the first 5 minutes after injection. The peak response occurred by 20 minutes and was completed by about 30 minutes. A substantial natriuresis, diuresis, and an increase in osmolar clearance occurred. The changes in potassium excretion, Ccr, and CH2O were variable, with the mean data showing no significant change. In contrast to the MA, MV produced only a small transient effect. The renal response to HA was essentially the same as that to

### Table 1
The Time Course of the Renal Response to an Intravenous Injection of Monkey Atrial Extract

<table>
<thead>
<tr>
<th>Time After Injection</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>40 min</th>
<th>50 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccr (ml/min)</td>
<td>7.8 ± 1.11</td>
<td>9.07 ± 1.14</td>
<td>6.37 ± 0.65</td>
<td>7.17 ± 1.7</td>
<td>6.58 ± 0.61</td>
<td>6.94 ± 1.1</td>
</tr>
<tr>
<td>UMV (μEq/min)</td>
<td>12.86 ± 3.44</td>
<td>104.98 ± 26.89</td>
<td>43.31 ± 9.36</td>
<td>36.08 ± 7.72</td>
<td>22.5 ± 3.76</td>
<td>19.8 ± 3.91</td>
</tr>
<tr>
<td>FNa (%)</td>
<td>1.21 ± 0.33</td>
<td>8.05 ± 1.94</td>
<td>4.74 ± 1.16</td>
<td>3.51 ± 0.61</td>
<td>2.36 ± 0.43</td>
<td>2.06 ± 0.41</td>
</tr>
<tr>
<td>UXV (μEq/min)</td>
<td>3.18 ± 1.14</td>
<td>6.65 ± 0.98</td>
<td>7.53 ± 1.0</td>
<td>4.31 ± 0.87</td>
<td>5.08 ± 1.07</td>
<td>4.77 ± 1.32</td>
</tr>
<tr>
<td>C4O (ml/min)</td>
<td>-0.11 ± 0.02</td>
<td>-0.01 ± 0.07</td>
<td>0.08 ± 0.09</td>
<td>-0.12 ± 0.01</td>
<td>-0.14 ± 0.02</td>
<td>-0.12 ± 0.02</td>
</tr>
<tr>
<td>Caw (ml/min)</td>
<td>0.2 ± 0.02</td>
<td>0.66 ± 0.16</td>
<td>0.92 ± 0.19</td>
<td>0.44 ± 0.07</td>
<td>0.37 ± 0.07</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>0.12 ± 0.02</td>
<td>0.65 ± 0.18</td>
<td>0.94 ± 0.26</td>
<td>0.35 ± 0.08</td>
<td>0.26 ± 0.06</td>
<td>0.19 ± 0.04</td>
</tr>
</tbody>
</table>

Values shown are means ± SEM; number of observations = 5. Ccr = creatinine clearance; UMV = sodium excretion; FNa = fractional sodium excretion; UXV = potassium excretion; C4O = free water clearance; Caw = osmolar clearance; V = urine volume.

* The control was taken as the average of the two periods before the extract injection.
† Statistically significant at P < 0.05.
‡ Statistically significant at P < 0.01.

### Table 2
The Time Course of the Renal Response to an Injection of Human Atrial Extract

<table>
<thead>
<tr>
<th>Time After Injection</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>40 min</th>
<th>50 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccr (ml/min)</td>
<td>8.02 ± 1.75</td>
<td>9.09 ± 1.62</td>
<td>8.22 ± 1.71</td>
<td>8.18 ± 1.8</td>
<td>7.99 ± 1.76</td>
<td>7.98 ± 1.76</td>
</tr>
<tr>
<td>UMV (μEq/min)</td>
<td>19.56 ± 5.47</td>
<td>105.24 ± 18.93</td>
<td>73.52 ± 16.94</td>
<td>50.51 ± 10.71</td>
<td>39.09 ± 5.58</td>
<td>30.63 ± 4.35</td>
</tr>
<tr>
<td>FNa (%)</td>
<td>1.72 ± 0.37</td>
<td>9.99 ± 2.68</td>
<td>7.83 ± 0.77</td>
<td>5.78 ± 2.24</td>
<td>4.55 ± 1.55</td>
<td>3.26 ± 0.86</td>
</tr>
<tr>
<td>UXV (μEq/min)</td>
<td>5.86 ± 2.51</td>
<td>12.42 ± 2.95</td>
<td>13.73 ± 4.39</td>
<td>9.19 ± 1.79</td>
<td>7.77 ± 1.97</td>
<td>8.67 ± 2.04</td>
</tr>
<tr>
<td>C4O (ml/min)</td>
<td>-0.14 ± 0.02</td>
<td>-0.13 ± 0.05</td>
<td>-0.06 ± 0.06</td>
<td>-0.15 ± 0.04</td>
<td>-0.18 ± 0.04</td>
<td>-0.17 ± 0.03</td>
</tr>
<tr>
<td>Caw (ml/min)</td>
<td>0.28 ± 0.06</td>
<td>0.95 ± 0.14</td>
<td>1.01 ± 0.19</td>
<td>0.75 ± 0.15</td>
<td>0.57 ± 0.1</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>0.14 ± 0.03</td>
<td>0.82 ± 0.14</td>
<td>0.97 ± 0.23</td>
<td>0.60 ± 0.15</td>
<td>0.40 ± 0.08</td>
<td>0.39 ± 0.04</td>
</tr>
</tbody>
</table>

Values shown are means ± SEM; number of observations = 5. For abbreviations, see Table 1.

* The control was taken as the average of the two periods before the extract injection.
† Statistically significant at P < 0.05.
‡ Statistically significant at P < 0.01.
MA. Figure 1 compares the natriuretic, and Figure 2 the calciuretic, responses to the injection of atrial extract, furosemide and chlorothiazide. For Figure 2, the human and monkey results were pooled, since the n for each was small but the response was the same. The natriuretic and calciuretic response to the atrial extract and furosemide were similar, whereas chlorothiazide produced a more prolonged natriuresis and no significant effect on calcium excretion. There was no consistent change in blood pressure in response to either the extracts or diuretics.

Discussion

The human and monkey atrial crude extract caused a significant natriuresis and calciuresis without a significant change in GFR. Others (de Bold et al., 1981; Trippodo et al., 1982; Keeler, 1982) have reported a significant rise in sodium excretion following the injection of rat or rabbit atrial extract, but they have also reported a significant decline in mean arterial blood pressure after atrial extract injection (de Bold et al., 1981) or after injection of either atrial or ventricular extract (Keeler, 1982). We did not notice any consistent change in blood pressure. The reason for this discrepancy could be species differences, since—when we injected the extract into rats—a significant decline in arterial pressure occurred.

The secretory granules of the atrial myocytes were first noticed in 1956 and afterward reported by many investigators (Kish, 1956; de Bold et al., 1978; Cantin et al., 1979). These granules were found to be a site of storage for a protein which may contain tryptophan and sulfur containing amino acids (de Bold et al., 1978). Furthermore, the studies of de Bold (1982) and Garcia et al. (1982) suggest that the granules are probably the storage site for atrial natriuretic factor. Although no one has shown a physiological role for these granules, it is tempting to hypothesize that they play an important role in the renal handling of sodium. However, there is little information available concerning factors which may control release of their contents. Since the granules which apparently contain the natriuretic factor are found in the atria, it has been suggested that the extent of atrial stretch is a determinant of the release of the atrial natriuretic factor (Sonnenberg et al., 1981). However, this would not appear to be true for the primate, since, in the primate atrial, distension has no significant effect on renal function (Cornish and Gilmore, 1982).

It has been noted since 1927 that profuse urine production is associated with paroxysmal atrial tachycardia (PAT) (Luria, 1971; Luria et al., 1966). In addition, a significant saluresis was found in every reported case for which urinary data exist (Kinney et al., 1974). Since PAT is associated with an increased atrial pressure, it has been suggested that the diuresis of PAT is the result of stimulating atrial receptors. However, the demonstration that atrial receptors in the primate are very insensitive (Zucker and Gilmore, 1975), and that stretching the primate atrium has little effect on renal function (Cornish and Gilmore, 1982), would indicate the need for a different explanation for the natriuresis and diuresis of PAT. It is tempting to speculate that the atrial factor is responsible. Against this possibility is the study of Goetz and Bond (1973), in which it was found that atrial tachycardia in the heart-blocked dog did not induce a natriuresis or diuresis, whereas a tachycardia produced by sequential AV pacing did. Since sinoaortic denervation prevented the natriuresis and diuresis, they concluded that
arterial baroreceptors were involved, rather than a mechanism(s) involving the atria. The results obtained by Goetz and Bond (1973) may reflect a species difference, since, in the human, there is no relation between the renal responses to PAT and ventricular rate.

The central nervous system is not necessary for the action of the natriuretic hormone, since atrial extract caused natriuresis in headless rats maintained at normal blood pressure with epinephrine and vasopressin infusion (Trippodo et al., 1982). Keeler (1982) found that atrial extract acts directly on the kidney by a mechanism which is independent of prostaglandin synthesis. Throckmorton and Gilmore (1983) found that atrial extract, unlike furosemide, does not inhibit short circuit current in the frog cornea even after adding plasma to it, which may indicate that the atrial extract causes the intrarenal release of another natriuretic substance, or that it undergoes intrarenal conversion to an active form. Sonnenberg and Cupples (1981) and Briggs et al. (1982) concluded that atrial extract acts by inhibiting sodium reabsorption in the medullary collecting duct.

The present study is the first in which calcium excretion was measured following atrial extract injection. As was reported previously by Hanson and co-workers (1982), we found that furosemide but not chlorothiazide caused a significant calciuresis which was, in magnitude and pattern of response, similar to the calciuresis following atrial extract injection. Although both furosemide and chlorothiazide have carbonic anhydrase inhibitory activity and thus can potentially alter proximal tubule reabsorption, the major site of action of furosemide is believed to be the thick ascending limb of Henle’s loop and the major site of action of chlorothiazide the distal convoluted tubule (Reineck and Stein, 1981). The observation that atrial extract produced a pattern of electrolyte excretion like that of furosemide suggests that atrial extract has a mechanism of action similar to that of furosemide. Finally, this study demonstrates that the atrial natriuretic factor is active in an animal other than the rat, giving support to the notion that the factor is active across species.

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References


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