Partially Purified Human and Rat Atrial Natriuretic Factor

NICK C. TRIPPODO, PH.D., ALLAN A. MACPHEE, PH.D., AND FRANCIS E. COLE, PH.D.

SUMMARY Specific granules of rat atrial myocytes contain a potent fast-acting atrial natriuretic factor (ANF) and have been observed in all mammalian atria thus far examined. In this study, using a rat assay we found ANF in rat, rabbit, dog, baboon, and man. The ANF's from these species induced the characteristic diuresis and natriuresis of rapid onset and decay. The human and rat ANF's were acid-extractable, heat-resistant, and trypsin-sensitive, suggesting that both are peptides. When fractionated on Sephadex G-75, ANF eluted at approximately 1.4 to 2.9 elution volume/void volume. The human and rat ANF dose-response relationships showed that changes in urinary sodium excretion ($\Delta U_{\text{Na}}/V$) were linearly related to log mg protein injected per kg rat assay weight ($r^2 = 0.9853$ and 0.9966, respectively). The minimum and maximum responses to rat ANF on the average were 14 ± 3 and 409 ± 51 μEq Na/kg-10 min (± SE) respectively. The $\Delta U_{\text{Na}}/V$ of rats given a second injection of ANF was not altered by a first injection. Furosemide induced a similar but slightly longer diuretic, natriuretic, and kaliuretic response pattern than that of ANF. Nevertheless, a classical four-point log dose-response analysis revealed that under certain conditions furosemide could be a suitable standard against which the potencies of unknown preparations of ANF can be compared. The relative potency of the partially purified rat ANF used in this study was 0.654 mg furosemide/mg protein (95% confidence limits, 0.378 to 1.135). The index of precision ($\lambda$) was 0.36. The results of this study suggest that mammalian ANF's, including that in man, share similar physiological and chemical characteristics. In addition, furosemide was found to be a suitable standard against which the potencies of unknown preparations of ANF can be compared. (Hypertension 5 (supp I): I-81–I-88, 1983)

KEY WORDS rabbit • dog • baboon • gel filtration • bioassay • log dose-response curve • furosemide

ACCORDING to current hypotheses, a putative circulating sodium-transport inhibitor or natriuretic hormone may be important in the control of renal sodium excretion and also may play a role in the development of genetic and some experimental forms of hypertension. Recently, de Bold et al. reported that a powerful, rapidly acting, natriuretic factor can be extracted from rat atrial myocardial tissue. This factor, which is referred to as the atrial natriuretic factor (ANF), appears to be a polypeptide that is stored within the specific granules of the atrial myocytes. Work from our laboratory confirmed the existence of this substance in rat atria and extended the characterization of its chemical and physiological properties. So far, ANF does not seem to be the small (< 1,000 daltons) sodium-transport inhibitor that is currently studied in other laboratories, nor does it have the same slow time-course of action of these other factors.

Much more work is needed not only to determine whether ANF plays a role in the normal physiological control of sodium excretion, but also to ascertain if it is involved in the pathophysiological mechanisms of hypertension. It also remains to be determined if ANF has any relationship to the small circulating sodium-transport inhibitor. ANF will have to be purified and a sensitive method developed to measure its concentration in blood and possibly urine.

Since it is also important to learn whether similar natriuretic factors can be extracted from the atria of mammals other than the rat, in this study we examined the possible existence of natriuretic activity in atrial extracts from rat, rabbit, dog, baboon, and man. In addition, since the rat assay is currently the only available means for measuring the activity of ANF, we endeavored to establish its quantitative characteristics by determining the dose-response relationship, the maximum and minimum responses, and by analyzing the data using a well established design for hormonal bioassays.

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Methods

Preparation of Extracts

Human atrial tissue was collected from adult patients undergoing cardiac bypass surgery for coronary artery disease at the Ochsner Foundation Hospital. Approval was obtained from the hospital’s Clinical Investigations Committee to collect small fragments, about 200 mg from each patient, of the right atrial appendage that the surgeons routinely remove for the placement of an extracorporeal circuit catheter. The atrial tissue was placed in 5 ml of 1.0 M acetic acid and stored at −90°C for up to 6 weeks before it was used for preparation of atrial extracts.

Atrial tissues were obtained from adult animals killed by decapitation or by high doses of sodium pentobarbital or ether. Hearts were taken from male Wistar and Sprague-Dawley rats (approximately 150 to 200 g), male New Zealand rabbits (1.8 to 3.2 kg), male and female dogs (14.7 to 17.8 kg), and female baboons (approximately 4 to 5 kg). Both right and left atria were dissected and washed in cold 0.9% NaCl. The atria then were used immediately for preparation of atrial extract or were stored at −90°C for up to 6 weeks before use.

For preparation of extract the atrial tissue was blotted to remove any excess fluid and weighed. Except for the rabbit atria, which were prepared as described below, the atrial tissue was homogenized with a Brinkman Polytron in 1.0 M acetic acid (10 ml/g). After centrifugation at 12,000 g for 10 minutes, the pellet was rehomogenized in five volumes of 1.0 M acetic acid and the combined supernatants were lyophilized and stored at −90°C.

The lyophilizate of the acid extracts formed a dry spongy material which required another extraction process before chromatography by gel filtration. Two different sized columns of Sephadex G-75 were used for gel filtration, 1.5 x 85 and 2.5 x 80 cm, both equilibrated in 0.5 M acetic acid. When lyophilizates were prepared for the smaller diameter column, material representing an original acid-extraction volume of 6–8 ml was homogenized with a Polytron in 2.0 ml of 0.5 M acetic acid and centrifuged at 12,000 g for 10 minutes. The pellet was rehomogenized in 1.0 ml acetic acid and centrifuged. The combined supernatants were placed on the column. For the larger diameter column, material representing an original acid-extraction volume of 25 to 30 ml was homogenized in 6 ml of 0.5 M acetic acid and centrifuged. The pellet was rehomogenized in 6 ml acetic acid and centrifuged. The combined supernatants were placed on the column. The smaller and larger columns were eluted with 0.5 M acetic acid at flow rates of 0.6 and 1.2 ml/min, respectively, as determined by a Technicon roller pump. The absorption of the eluates was monitored at 280 nm and fractions were collected at 4°C. The fractions were lyophilized, stored at −90°C, and dissolved in Dulbecco’s phosphate buffered saline of pH 7.2 (PBS) for assay for natriuretic activity, as described below. Lyophilizates of pooled active fractions are referred to as partially purified extracts. The columns of Sephadex were calibrated with known proteins as described previously.14

Human atrial extracts were prepared by homogenizing the tissue in 10 volumes of PBS, placed in boiling water for 10 minutes and centrifuged at 12,000 g for 10 minutes. The supernatants were assayed on the same day that the extracts were prepared.

Bioassay

Male Sprague-Dawley rats (148 to 219 g), maintained on Purina laboratory chow (containing 0.4% sodium and 1% potassium) and water ad libitum, were used to assay for natriuretic activity as described previously.14 Briefly, the rats were anesthetized with ether, and catheters were placed in the trachea, urinary bladder, and in the left femoral vein and artery. After a 30-minute stabilization period, control urine was collected during three 10-minute periods. Extracts were injected intravenously over 1 to 3 minutes at 4 ml/kg, and urine was collected for two 10-minute periods after the start of injection. Mean arterial pressure was monitored continuously with a Statham transducer and a Grass recorder. Urine volume was determined by weight, and sodium and potassium concentrations were measured by flame photometry. All urine samples were diluted with 0.5 ml deionized water before analysis.

Dose-Response Curve and Treatments

To obtain a large homogeneous batch of partially purified rat atrial extract, lyophilizates of pooled active fractions from approximately 30 batches of extracts were prepared and stored at −90°C over a period of about 4 months. The combined lyophilizates were then dissolved in 13 ml of 0.5 M acetic acid and divided into 13 1-ml aliquots. The aliquots were lyophilized and stored at −90°C in flame-sealed glass containing nitrogen instead of air. After preliminary experiments allowed an estimation of the amount of natriuretic activity contained in each flask, some of the aliquots were redissolved in 0.5 M acetic acid, and further divided into four additional equal aliquots that were lyophilized and stored under nitrogen at −90°C as before. Using the original 1/13 aliquot dissolved in 2.4 ml PBS as the basis, we made the following serial dilutions in PBS: 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64. Only the amount that could be assayed in 1 day was dissolved in PBS for assay. Ten assays were done on each of the above dilutions using one Sprague-Dawley rat per assay. A small portion of each injectate was saved for protein analysis by the method of Lowry et al.,16 so that the dose-response curve could be expressed in terms of mg protein injected/kg assay weight.

To determine if two injections of ANF could be given to each rat without the first injection interfering with the response to the second injection, two groups of male Sprague-Dawley rats (154–190 g) were prepared for bioassay as described above. Each rat first received a high (2.4 ± 0.3 mg/kg) or low (0.8 ± 0.1 mg/kg) dose of lyophilized extract.
mg/kg) dose of the homogeneous partially purified rat atrial extract described above followed by two 10-minute periods of urine collection. The rats were allowed to stabilize for 20 minutes before the second 30-minute control period was begun and then an injection of the opposite dose was given. Thus, one group of rats (n = 13) received the high dose followed by the low dose, and the second group (n = 12) received the low dose followed by the high dose.

To compare the potency of the rat ANF extract to a stable “standard preparation” by classical analysis, additional assays were done on three doses (0.2, 0.8, and 2.0 mg/kg) of furosemide (10 assays/dose). Furosemide (Lasix, 10 mg/ml ampuls) diluted in PBS to 0.05, 0.2, and 0.5 mg/ml was injected intravenously at 4 ml/kg.

To determine if ANF was susceptible to heat or proteolysis, partially purified human and rat atrial extracts each were divided into three 1-ml aliquots, which were untreated, placed in a boiling water bath for 10 minutes, or incubated with a suspension of insoluble bovine pancreatic trypsin-agarose (Sigma).

One ml of the trypsin-agarose suspension contained 43 units and one unit hydrolyzed 1.0 μmol of BAEE per minute at pH 8.0 at 30°C. The partially purified extracts, dissolved in 1 ml of PBS, were mixed and incubated with 0.1 ml of a suspension of PBS-washed insoluble trypsin-agarose at 37°C for 1 hour. The trypsin-agarose was removed from the extracts by centrifugation before the extracts were injected into the assay rats.

Statistics

To determine an appropriate response to use in the dose-response relationship, several variables were analyzed by linear regression using the method of least-squares. The variable that had the least residual or proportion of unexplained variance (1-coefficient of determination) in relation to log dose was taken as the most appropriate response. To obtain the approximate minimum response from the dose-response relationship the responses resulting from the two lowest doses given were analyzed for significance of difference from “0 response” by the paired t test and for significance of difference from each other by the unpaired t test. Since three comparisons were made in this analysis, the critical value for the t statistics was determined by the Bonferroni method. In the experiments where two injections of partially purified rat atrial extract were given to the assay rats, a two-way analysis of variance was performed to determine whether the first injection altered the responsiveness of the rats to the second injection. Statistical analysis of the four-point parallel-line assay was performed according to classical methods as modified by Cox. A p value of 0.05 or less was considered significant for all statistical analyses.

Results

Injection of the extracts from rat, rabbit, dog, baboon, and human atrial tissue caused marked increases in urine volume (UV) and urinary sodium excretion (UNaV) and moderate increases in urinary potassium excretion (UKV). The pattern of the diuretic, natriuretic, and kaliuretic responses (i.e., the onset times, durations, and relative magnitudes) were similar for all extracts from the various species. As typified by the renal reaction to partially purified rat atrial extract shown in figure 1, the response reached a peak within the first 10 minutes after injection and decreased rapidly toward control by the second 10 minutes. This rapid onset and decay and the large magnitude (i.e., up to a 30-fold increase during the first 10 minutes) of the natriuretic response are characteristics that distinguish this natriuretic factor from others. Another characteristic of partially purified atrial natriuretic factor (ANF) is that the kaliuretic effect is less in magnitude than the natriuretic effect (fig. 1). Figure 2 shows the time course of the natriuretic responses that resulted from injection of partially purified extracts of rabbit, dog, baboon, and human atria.

Only transient small decreases in mean arterial pressure were observed after injection of the extracts (i.e.,
The greatest decrease in mean arterial pressure resulted from injection of crude extracts (in this study the rabbit extracts were not processed by gel filtration and are referred to as crude extracts) or from injection of the gel-filtration fractions containing mostly substances less than about 3000 daltons (which had no natriuretic activity). There were no consistent differences among partially purified extracts from the different species with respect to their effects on arterial pressure.

The patterns of natriuretic activity eluting from Sephadex G-75 were similar for both human and rat atrial extract (figs. 3 and 4). The greatest natriuretic activity eluted at 1.4–1.8 Ve/Vo (elution volume/void volume) with moderate amounts trailing at 2.7–3.0 Ve/Vo. These elution volumes corresponded to a molecular weight spread of approximately 44,000 to 3100 daltons. For subsequent experiments utilizing human and rat atrial extracts, the fractions eluting from Sephadex G-75 at approximately 1.4–3.0 Ve/Vo were pooled and lyophilized.

To determine an "appropriate response" for the dose-response relationship using partially purified rat atrial extract, the following variables were analyzed by linear regression in relation to log dose (log mg protein injected per kg body weight of the assay rat): %ΔUNV10min, %ΔUNV20min, ΔUNV10min, ΔUNV20min, ΔUNV10min/ΔUNV20min, and ΔUNV20min/ΔUV20min. The changes in UNV10min and UNV20min were determined from the averages of three 10-minute control periods and the 20-minute changes were determined from the sums of the two 10-minute control periods immediately preceding injection of the extracts. From these analyses it was obvious that the lowest and highest doses used were not on the linear portion of the log dose-response curve (fig. 5) and therefore comparisons of the above variables were made for the four intermediate doses. The variable that showed the highest coefficient of determination (r² = 0.9966) with log dose and thus the least residual or proportion of unexplained variance was ΔUNV10min. Hence, ΔUNV (µEq/kg-10 min) was taken as the most appropriate response.

Injection of 0.23 ± 0.01 mg/kg partially purified rat atrial extract caused UNV to increase by 14 ± 3 µEq/
Figure 4. The average gel filtration (Sephadex G-75) results from two batches of human atrial extract. The greatest natriuretic activity was found at 1.6 to 2.9 $V_e/V_o$, representing a molecular weight spread of 30,000 to 3100 daltons.

kg·10 min. This response was significantly different from zero ($p < 0.01$) and was also different ($p < 0.02$) from the response (1 ± 3 $\mu$Eq/kg·10 min) that resulted from injection of 0.13 ± 0.01 mg/kg, which was not significantly different from zero (fig. 5, table 1). Therefore, a $\Delta U_{NaV}$ of 14 $\mu$Eq/kg·10 min was considered the minimum response measurable in this study with 99% confidence. Injections of 1.81 ± 0.10 and 3.15 ± 0.10 mg/kg of partially purified rat atrial extract caused $U_{NaV}$ to increase 409 ± 51 and 402 ± 62 $\mu$Eq/kg·10 min, respectively (table 1). The results suggested that, on the average, the maximum response to partially purified rat ANF was about 400 $\mu$Eq/kg·10 min.

An experiment was conducted to determine if the rats could be used for two injections of ANF. One group of rats received a high followed by a low dose, and another group received a low followed by a high dose, of partially purified rat atrial extract. Before the second injection in these rats, $U_{NaV}$ returned very close to the previous control levels. For example, in the rats that received the high then the low dose, the average $U_{NaV}$ during the first and second control periods was 21.0 ± 5.4 and 20.5 ± 4.9 $\mu$Eq/kg·10 min, respectively (mean ± se). In the rats that received the low then the high dose, the respective values were 20.2 ± 6.1 and 22.0 ± 4.0 $\mu$Eq/kg·10 min. The $\Delta U_{NaV}$ of the rats that received the high dose as the first injection was 424 ± 41 $\mu$Eq/kg·10 min (mean ± se) and was not significantly different from that (481 ± 44) of the rats that received the low dose as the second injection. The response of the rats that received the low dose as the first injection was 256 ± 56 $\mu$Eq Na/kg·10 min and was not significantly different from that (163 ± 40) of the rats that received the low dose as the second injection. These results indicate that the responsiveness of the assay rats to a second injection of partially purified rat atrial extract was not significantly altered by a prior injection of ANF and suggest that the assay rats can be used for at least two injections of preparation.

Table 1. Summary Data of Rat ANF Dose-Response Curve

<table>
<thead>
<tr>
<th>Dose (protein) (mg/kg)</th>
<th>Response (Δ$U_{NaV}$10 min) (μEq/kg·10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.132 ± 0.010</td>
<td>1 ± 3</td>
</tr>
<tr>
<td>0.230 ± 0.014</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>0.529 ± 0.034</td>
<td>160 ± 51</td>
</tr>
<tr>
<td>0.935 ± 0.039</td>
<td>264 ± 49</td>
</tr>
<tr>
<td>1.812 ± 0.100</td>
<td>409 ± 51</td>
</tr>
<tr>
<td>3.150 ± 0.101</td>
<td>402 ± 62</td>
</tr>
</tbody>
</table>

Values are means ± se.

Figure 5. Dose-response (upper graph) and log dose-response (lower graph) curves for a homogeneous batch of partially purified rat atrial extract. Means ± se are shown. The se for the lowest two doses was too small to be depicted on the graphs. At each dose, 10 assay rats (171 ± 2 g, mean ± se body weight for all 60 rats) were injected intravenously at 4 ml/kg. Each rat received only one injection.
The pattern of the renal excretory response to furosemide was similar to the response to partially purified rat atrial extract, except that the duration of action was slightly longer and the peak response sometimes occurred during the second 10 minutes after injection rather than the first. This made it difficult to choose which time period after injection to use as the "response" to furosemide. However, since there was greater variability (evidenced by larger standard deviations) in the $\Delta U_{Na}$V's measured only during the first 10 minutes as compared with those measured as a peak $\Delta U_{Na}$V per 10 minutes, the latter variable was chosen. In response to the three doses of furosemide (0.2, 0.8, and 2.0 mg/kg) the mean $\Delta U_{Na}$Vpeak was $84 \pm 15$, $269 \pm 48$, and $481 \pm 56 \mu$Eq/kg·10 min, respectively, and was linearly related to log dose with a $r^2$ of 0.9785. The $\Delta U_{Na}$Vpeak and $\Delta U_{Na}$V for the three doses of furosemide were $809 \pm 137$, $2403 \pm 355$, and $3875 \pm 406 \mu$Eq/kg·10 min, and $69 \pm 13$, $76 \pm 10$, and $120 \pm 17 \mu$Eq/kg·10 min, respectively.

To examine the possibility of using furosemide as a standard for future assays of ANF, a statistical analysis of a four-point parallel-line assay was conducted on the results from partially purified rat atrial extract and two doses of furosemide. The results are shown in table 2.

To determine if the dose-response relationship for human atrial extract was similar to that for rat atrial extract, three different doses of partially purified human atrial extract were given to seven assay rats. Injection of 1.87, 2.63, and 5.32 mg protein/kg assay weight caused $U_{Na}$V to increase by 45, 150, and 530 $\mu$Eq/kg·10 min, respectively (fig. 6).

Human and rat atrial extracts heated to 100°C did not lose natriuretic activity, whereas after the extracts were treated with trypsin, natriuretic activity was completely abolished (fig. 7).

### Table 2. Four-Point Log Dose-Response Assay of Rat ANF Using Furosemide as the Standard

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Standard (Furosemide)</th>
<th>Unknown (Rat atrial extract protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>2.0</td>
<td>0.529</td>
</tr>
<tr>
<td>2.0</td>
<td>0.2</td>
<td>1.81</td>
</tr>
<tr>
<td>Response ($\mu$Eq Na/kg·10 min)</td>
<td>Mean: 84, 481</td>
<td>160, 409</td>
</tr>
<tr>
<td></td>
<td>Total: 840, 4810</td>
<td>1600, 4090</td>
</tr>
</tbody>
</table>

Variation source

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>f.</th>
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<tbody>
<tr>
<td>Preparation</td>
<td>1</td>
<td>42,025</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>1</td>
<td>1,042,996,109</td>
<td>50,799</td>
<td></td>
</tr>
<tr>
<td>Divergence</td>
<td>1</td>
<td>535,681</td>
<td>0.249</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>774,586,160</td>
<td>21,516,282</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>1,872,977,975</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Relative potency = $0.654$ mg furosemide/mg protein
95% confidence limits = $0.378-1.135$ mg furosemide/mg protein

Common slope ($b$) = $412,541 \mu$Eq Na/kg·10 min per log (mg/kg)

$\lambda = \sqrt{\text{residual m.s.}/b} = 0.36$
Discussion

The results of this study show that natriuretic substances similar to the natriuretic factor previously found in the specific granules of rat atria also can be extracted from rabbit, dog, baboon, and, more important, human atria. Indeed, specific granules have been observed in all mammalian atria thus far examined, including humans. The natriuretic substances from these species showed the same rapid onset and decay of action that is characteristic of the rat atrial natriuretic factor (ANF). That is, the natriuretic and diuretic responses reached peaks within the first 10 minutes after injection and decreased almost to control by the second 10 minutes. Except for the rabbit ANF, which was not studied by acid extraction, they all were extractable in acid and shared similar chromatographic properties on Sephadex G-75. In addition, the human and rat ANF both were found to be heat-resistant and trypsin-sensitive, suggesting that both are peptides. These findings suggest that the atrial natriuretic factors studied in the various mammals thus far share similar chemical and physiological characteristics.

However, a more thorough comparison of the atrial natriuretic factors from the different species only can be carried out after greater purification of the substances has been achieved. For instance, question still remains as to whether there are multiple molecular forms of ANF. The observation that ANF activity elutes from Sephadex G-75 over a wide molecular weight range (about 3100 to 44,000) suggests the existence of two or more forms of ANF and/or the presence of aggregates. Furthermore, in the present bioassay system, the natriuretic response to atrial extracts of various species will be affected by species differences in sensitivity as well as by differences in the heart content of ANF.

Since the rat bioassay is currently the only available means for measuring the activity of ANF, it was important that it be fully characterized so that the extent of its usefulness could be evaluated. The changes in $U_{\text{Na}}$ after injection of six doses of partially purified rat atrial extract were analyzed by various means (i.e., $\% \Delta U_{\text{Na}}$ and $\Delta U_{\text{Na}}$ at 10 and 20 minutes and as ratios to $\Delta U_{\text{Na}}$). The responses resulting from the four intermediate doses were found to be a linear function of the log dose. Of these variables, the $\Delta U_{\text{Na}}$ during the first 10 minutes after injection was found to have the least residual or proportion of unexplained variance in relation to log dose, and was therefore taken as the most appropriate response.

The maximum response of the bioassay, on the average, was about 400 $\mu$Eq/kg·10 min, although individual rats showed much larger responses. Using a similar rat assay, de Bold reported a maximum response of about 4100 nEq/min per gram of kidney. If the pair of kidneys in the rats weighed 7.97 ± 0.18 g/kg (which is the mean ± SE measured in 15 of our assay rats), our maximum response converts to 5019 nEq/min per gram of kidney. This somewhat higher maximum response, as compared with de Bold’s, can be explained by the 10-minute test period used in our present study for urine collection after injection, as compared with the 20-minute test period used by de Bold. Since $U_{\text{Na}}$ decreases precipitously during the second 10-minute period, a $\Delta U_{\text{Na}}$ averaged over the longer time period obviously would be less than one measured only during the first 10 minutes. Hence, our 10-minute response was expected to be greater than the 20-minute response. The minimum response measurable with 99% confidence was estimated as 14 $\mu$Eq/kg·10 min, since this change in $U_{\text{Na}}$ was significantly different from 0 and also was significantly different from the response to the next lower dose which was not significantly different from 0.

Due to the large variability in biological response, most investigators employing bioassay systems use, if possible, a stable standard preparation against which the potencies of unknowns can be compared. When the response producing constituents in the standard and the unknown preparations are the same, the assay is referred to as an analytical dilution assay. When the response producing constituents in the two preparations are only qualitatively similar, the assay is referred to as a comparative dilution assay. Since a universal standard preparation of ANF is currently not available, we examined the possibility of using furosemide as a standard for use in a comparative dilution assay. Sonnenberg et al. presented evidence that ANF and furosemide may act on the renal tubules through a similar mechanism, although this is not a prerequisite for use of furosemide as a comparative standard.

Our results indicated that, although the diuretic, natriuretic, and kaliuretic response pattern to furosemide was similar to the response pattern to ANF, the duration of action of the higher doses was slightly longer. Thus, the peak response sometimes occurred during the second 10-minute period after injection rather than always in the first, as with ANF. Despite this difference in the time-course of action between furosemide and ANF, the four-point parallel-line analysis indicated that using furosemide as a standard is quite feasible if the peak $\Delta U_{\text{Na}}$·10 min is taken as the response for both ANF and furosemide (table 2). For instance, the nonsignificant f value of 0.2 for divergence suggested that the assumption of parallelism between the standard and unknown log dose-response curves was justified. The relative potency, $R$, was 0.654 (95% confidence limits, 0.38 to 1.14), and the index of precision ($\lambda$) was 0.36.

The results also suggest that each rat can be given at least two injections of ANF preparation without the first interfering with the response to the second. Thus, it should be possible to compare the potency of an unknown to that of furosemide in a single rat. Indeed, studies are in progress in our laboratory showing the feasibility of this type of analysis. Each rat is given an injection of unknown and an injection of furosemide, and the amount of ANF in the unknown is quantitated relative to the rat’s response to furosemide. Comparing the response to an unknown to the response resulting from injection of a standardized dose of furosemide in
individual rats improves precision considerably since the greatest variability arises from differences in overall natriuretic responsiveness among rats, i.e., between-rat variation.

Any statements regarding the possible role of ANF in the pathophysiology of hypertension would be entirely speculative at this time. It is still unclear if ANF for all natriuretic responsiveness among rats, i.e., between-rat variation.

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