Letters to the Editor

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ANP Bioactivity in Obese Hypertensives

To the Editor:

I write to comment on and to question features of the recent publication from Dessi-Fulgheri and colleagues that appeared in the February issue of *Hypertension* (Hypertension. 1999;33:658–662). The authors report that a bolus injection of ANP produces greater falls in blood pressure, greater increases in plasma cyclic GMP, and a greater suppression of plasma aldosterone that are all accompanied by increased natriuresis in obese subjects who have had a low-calorie diet for 4 days as compared with the response to ANP prior to caloric restriction.

Several difficulties arise with this report. First, the dose employed is unclear. The abstract employs 0.6 mg/kg. In “Results,” a dose of 0.6 μg/kg is reported, and then in “Discussion,” 0.6 mg/kg reappears. This 1000-fold difference in dose should be of some concern. Notably, even 0.6 μg/kg (over 50 μg as a bolus) is a large dose and cannot be considered as ranking among “low doses” as these authors suggest. A bolus injection of this dose will produce pharmacological levels of plasma ANP never reproduced in health or in any pathophysiological state. Certainly such an injection will not result in “changes in circulating ANP confined within the normal range” as these authors claim. This information was well established by the mid-1980s when it became clear that the plasma half-life of human ANP was a little under 3 minutes. Early reports from our group indicated that a 100-μg bolus resulted in plasma ANP levels in excess of 2500 pmol/L at 1 minute after intravenous injection, with an exponential fall in levels over the first 10 minutes, and a return to basal values of plasma ANP after 30 minutes (Yandle et al. Life Sci. 1986;38:1827–1833). This report has been repeatedly confirmed by other investigators over the last decade. Dessi-Fulgheri et al provide no measurements of plasma ANP prior to a stated peak level said to occur 30 minutes after peptide administration. This is quite implausible. It is notable that the protocol described in “Methods” states that plasma samples were taken at the time of ANP bolus (time 0) and 15, 30, 60, 90, and 120 minutes after ANP. However, only one time point is reported in the text. Furthermore, where other plasma variables are graphed (eg, Figure 2 shows plasma cyclic GMP concentration), the items depicted include 10, 20, and 30 minutes rather than 15 and 30 minutes.

The achieved levels of plasma ANP throughout the course of this experiment (including the first 15 minutes) are crucial to interpretation of its physiological or pathophysiological significance. Why have plasma concentrations not been measured in the first few minutes after injection when it is obvious they will be at their peak, and why are levels not depicted for all time points when measurements are claimed to have been made?

If these authors had wished to reproduce physiological or pathophysiological shifts in plasma ANP (which would be the appropriate approach to this experiment), then doses in the order of 2 or 3 μg/kg/minute (ie, in the order of 10 μg delivered over an hour to a 70 kg subject) would be appropriate (Richards et al. J Clin Endocrinol Metab. 1988;67:1134–1139; Richards et al. Hypertension. 1989;14:261–268).

Finally, the biological effects of this 50-μg dose (assuming that the authors do mean 0.6 μg/kg) are implausibly small in terms of the natriuresis observed. A 100-μg bolus of ANP produces a several-fold rise in urinary excretion of sodium over the first 30 minutes after the injection in both normal volunteers and patients with hypertension, and some natriuretic effect is sustained out to at least 90 minutes post-injection (Richards et al. Lancet. 1985;1:545–549; Richards et al. Hypertension. 1985;7:812–817). The authors may have obscured some of the natriuretic effect by plotting data in 2 hour blocks; however, it is still notable that there is no significant natriuresis in the non-calorie restricted state, and this requires more explanation than is offered in the current paper.

These questions challenge the plausibility of this report and cast doubt on its reliability as an indicator of physiological or pathophysiological events.

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Response

In relation to the comments of A.M. Richards concerning our paper published in the February issue of *Hypertension*,1 we confirm that the dose of ANP bolus employed was 0.6 μg/kg, as correctly reported in the “Methods” and in the “Results” and not, as erroneously indicated in the “Abstract” and in the “Discussion,” 0.6 mg/kg. The exact timing of blood sampling was 30 minutes and then, as reported in the figures, 0, 10, 20, 30, 60, 90, and 120 minutes after ANP bolus, not including the 15-minute blood sampling. We apologize to the readers of *Hypertension* for these errors that were not detected during proof correction, and we thank Dr Richards for noticing them.

Considering the other point underlined by Dr Richards, we agree that an earlier determination of plasma ANP levels after bolus injection would have better represented the peak level and that, probably, the ANP plasma levels in the first minutes after bolus would have been higher than those measured afterward. However, the aim of our study was not to evaluate precisely the absolute effects of an ANP bolus (which was well studied in the past as also mentioned by Dr Richards) but to compare the biological effects of the same pro-kilo dose of ANP before and after a low-calorie diet in obese patients since previous studies of our group demonstrated that NP-receptor C (clearance receptor) is very abundant in adipose tissue, particularly in obese hypertensives, and its gene expression is dramatically downregulated, in the rat, by fasting.2–5 Accordingly, our hypothesis was that both the adipose mass and its variations should have a role in modulating the biological effects of ANP. Although it is likely that the peak plasma level of ANP was reached earlier than evaluated by us, its biological effects are not strictly confined around the minutes near the peak, and our study was precisely aimed to evaluate any difference in the duration of the “biological effect” of ANP due to low-calorie diet in obese hypertensives. In addition, a previous study demonstrated that with continuous infusion of ANP, the effects on diuresis and natriuresis were transient and evanescent and not maintained during prolonged infusion.6

We confirm our interpretation on the role of adipose mass, through the action of the clearance receptor, in the modulation of the biological activity of natriuretic peptides. In previous studies on ANP infusion, the role of body weight was not taken into account and the majority of such studies were performed in subjects with normal body weight or body weight was not mentioned. This problem might justify some discrepancies between our study and previous studies and in particular the lack of a significant natriuretic effect when the injection of ANP was performed before diet. However, considering, as suggested by Dr
Richards, natriuresis every 30 minutes in the 2 hours before and after ANP bolus (and not as the hourly mean), we found a significant natriuretic effect of ANP bolus also in basal condition (from 4.6±0.4 mmol/30 min to 6.9±0.5 mmol/30 min, P<0.05). This natriuretic effect, similar to that found in other studies, was, however, transient and evanescent, and the natriuretic effect disappeared after the first 30 minutes. After diet, on the contrary, the diuretic and natriuretic effect was more prolonged as was the blood pressure lowering effect. The role of low-calorie diet, in modulating the activity of ANP, is also demonstrated by the finding that, after diet and independently from ANP administration, the urinary excretion of cGMP was significantly increased. Our hypothesis on a peripheral modulation of the physiological effects of ANP is strongly supported by a recent paper by Matsukawa et al. These authors demonstrated that in “knock out” mice for the gene for the clearance receptor of natriuretic peptides the biological effects of ANP (such as cGMP excretion) and its half life are increased in comparison with the “wild-type” mice despite the fact that plasma ANP levels were comparable.

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