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Short Communication

Effect of Atrial Natriuretic Factor on Skin Microcirculation Versus Skeletal Muscle Blood Flow

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Summary: The response of the skin microcirculation and of forearm skeletal muscle blood flow to infusion of α -human (99-126) atrial natriuretic factor (ANF) into the brachial artery was investigated in 15 young (18-25 years) healthy volunteers in a double-blind, randomized, placebo-controlled study. The forearm blood flow (FBF) was measured with venous occlusion plethysmography, and the skin flux was measured by using laser Doppler fluxmetry (LDF). Dose-response curves were made using increasing dosages of ANF: 1, 10, and 100 ng/min/dl forearm volume. The FBF showed a significant, dose-dependent increase during ANF infusion, averaging $107 \pm 22\%$ during the highest ANF dosage, as compared with $-5 \pm 9\%$ during placebo ($p < 0.001$). For the LDF, these

numbers were 34 ± 21 and $-6 \pm 10\%$, respectively (NS). In two subgroups of subjects, the effect of ANF on microvascular reactivity was assessed by registering the vasoconstrictor response to cold exposure ($n = 7$) and the vasodilator response to arterial occlusion ($n = 7$). ANF did not change the microvascular response to these stimuli. ANF induces a dose-dependent increase in skeletal muscle BF without a relevant response in the skin microcirculation. ANF does not play an important role in the regulation of skin perfusion. **Key Words:** δ -Human (99-126) atrial natriuretic factor—Skin microcirculation—Laser Doppler flux—Forearm blood flow—Plethysmography.

The hormonal system of atrial natriuretic factor (ANF) comprises a family of polypeptides of which the 28-amino acid (99-126)-human ANF is the major circulating form (1). ANF is considered to contribute essentially to the control of circulatory homeostasis in physiological as well as in pathophysiological conditions (2,3). ANF exerts important effects on the renin-angiotensin system and (para)-sympathetic nervous system, in addition to having direct vasodilator and renal actions (4,5). The marked vasodilator effects in vitro and in normal subjects have been described especially in large vessels (6,7). Some studies show controversial results of the vasodilator effect of ANF; e.g., data suggest that ANF may cause selective vasodilation of the kidney (8), contrary to data indicating no alteration in renal blood flow (9). ANF has been demonstrated

to increase dose dependently both skin perfusion and muscle blood flow (BF). In these particular studies, ANF was administered intravenously in high dosages (10), and intraarterially by infusion of supraphysiological (pharmacological) dosages (11). Previously we demonstrated that the systemic ANF administration evokes a slight vasodilation in the skin microcirculation of healthy elderly humans (12). We have now studied the effect of exogenous ANF on skin BF as indicated by laser Doppler flux (LDF: capillary and shunt BF in the skin) versus the effects of ANF on the skeletal muscle forearm BF (FBF) indicated by venous occlusion strain-gauge plethysmography. Because in vitro studies in rats have shown that ANF reverses α -adrenoceptor-mediated vasoconstriction (13), we also investigated the effect of ANF on microvascular reactivity.

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SUBJECTS AND METHOD

The study was approved by the local ethics committee. A group of healthy young volunteers ($n = 15$), recruited from the general population was submitted to clinical examination before admission to the study. Physical examination and laboratory investigation showed no abnormalities. None of the subjects used any drugs (oral contraceptives included) or abused alcohol. The subjects were instructed to abstain from smoking and not to drink any coffee or tea for 24 h preceding the experiment.

Study design

The study was performed during a 4-h immobilization period in a climatized room (temperature \pm SD: $22.6^\circ \pm 0.2^\circ\text{C}$; $n = 15$). The subjects were allowed to eat a light meal, but not within 2 h before the test. The tests were performed after an equilibration period of at least 45 min with the subjects in the supine position.

The left brachial artery was cannulated with a 20-gauge Angiocath cannula (Deseret Medical, Becton Dickinson, Sandy, U.S.A. UT, U.S.A.) for drug infusion (automatic syringe infusion pump, type STC-521, Terumo, Tokyo, Japan) as well as for blood pressure (BP) monitoring (Hewlett Packard monitor, type 78353B, Hewlett Packard GmbH, Böblingen, Germany). Because the dosages of all drugs were calculated per deciliter of forearm volume, this volume was measured in all participants by water displacement. The subjects remained supine throughout the 4-h study period.

During the study, systolic and diastolic BP (SBP, DBP) and mean arterial pressure (MAP) were recorded intra arterially. Heart rate (HR) was monitored continuously by ECG registration. FBF was measured by venous occlusion plethysmography with mercury-in-rubber strain gauges (Loosco BVP 96 Hoekloos, Amsterdam, Holland). A cuff was placed around the left arm, proximal to the strain gauge. Venous occlusion was attained by inflation of the cuff to 40 mmHg. Each FBF recording period took 4 min, during which time nine recordings were obtained. During the measurements, the left arm was maintained at heart level (14). Digital skin blood flux was recorded continuously with laser Doppler fluxmetry (Periflux Pf 1d, Perimed, Sweden). The laser light penetrates to a maximum depth of 1.5 mm depending on skin composition. The Doppler flux reflects skin microcirculation, including nutritional capillaries and arteriovenous shunt flow (15,16). The flux was expressed in perfusion units (PU). To the center of the left distal palmar tip of the third finger, a 90° angular probe was attached by a double-sided adhesive ring. Before each experiment, an electrical calibration procedure was performed according to the manual; i.e., the probe was placed in a calibration clip on zero surface, and the recorder pen was moved to zero on the recorder. To correct flux registration for spontaneous vasomotion, biological zeroing was performed during total occlusion of the finger circulation for 5 min. Skin temperature was measured in degrees Centigrade (Thermocouple, Ellab Instruments, Denmark) with the probe attached to the palmar side of the middle phalanx on the third finger. All parameters were measured during the several infusions of the study.

ANF or placebo was infused into the brachial artery in random order. Three increasing dose steps were administered: 1, 10, and 100 $\mu\text{g}/\text{min}/\text{dl}$ forearm tissue for 10 min

per dose. To evaluate the effect of ANF/placebo on the microvascular reactivity, the highest dose of ANF or placebo infusion was continued to perform a finger cooling test in a subgroup of 7 subjects or a post occlusive reactive hyperaemia test in 7 other subjects. In the finger cooling test, finger skin temperature and LDF were measured during a 25-min period, beginning with a 5-min cooling procedure in water of 15°C , as described previously (17). The postocclusive reactive hyperemia test was performed by inflating a finger cuff to 230 mm Hg for 5 min (18). After the cuff was released, the LDF was registered for 10 min. After a new equilibration period of 45 min, the same procedure was repeated, but with infusion of the alternate agent, placebo or ANF.

Drugs

α -Human (99-126) ANF was purchased from Bissendorf Peptide GmbH, Wedemark, Germany. Immediately before use, ANF was diluted in a 48-ml Haemaccel (Hoechst) solution. Haemaccel is a plasma-expanding solution and was used for placebo infusion, as well as for dissolving ANF. Results of pilot studies showed that dissolving iodinated ANF in Haemaccel solution resulted in a 20% loss of radioactivity to the infusion system as compared with a 50% loss when ANF was dissolved in 0.9% in saline solution, as we reported previously (12).

Data

All results are mean \pm SEM unless indicated otherwise. LDF was averaged over the last 5 min of each ANF infusion dosage. FBF was calculated as the mean of all registration during the last 4 min of each dose.

Statistical analysis

To determine the effects of ANF infusion within each group of subjects, we compared the absolute as well as percentage ANF-induced changes with corresponding placebo observations for each infusion rate by analysis of variance (ANOVA) with repeated measures. In case of a significant ANF effect, Fisher PLSD tests (protected least significant difference) were used as post hoc tests to analyze the differences between baseline and the several ANF dosages and also for the analysis of the finger cooling tests and the postocclusive reactive hyperemia tests. Differences were considered statistically significant at $p < 0.05$.

RESULTS

In all, 9 males and 6 females participated in the study. Their mean (\pm SD) age, body mass index, SDB/DBP, and HR was 25 ± 4 years, 22 ± 2 kg/m^2 , $125 \pm 6/73 \pm 6$ mm Hg, and 74 ± 12 beats/min, respectively. The serum creatinine concentrations averaged 72 ± 9 μM .

Effects of ANF on baseline parameters ($n = 15$)

Figure 1 shows the absolute changes from baseline induced by graded infusion of ANF or placebo for the FBF as well as for the LDF. The baseline FBF was 2.1 ± 0.3 ml/min/dl in both the placebo and the ANF test. Corrected for the changes induced by placebo, the FBF increased 0.2 ± 0.3 during the lowest, 0.8 ± 0.3 during the middle, and 2.1

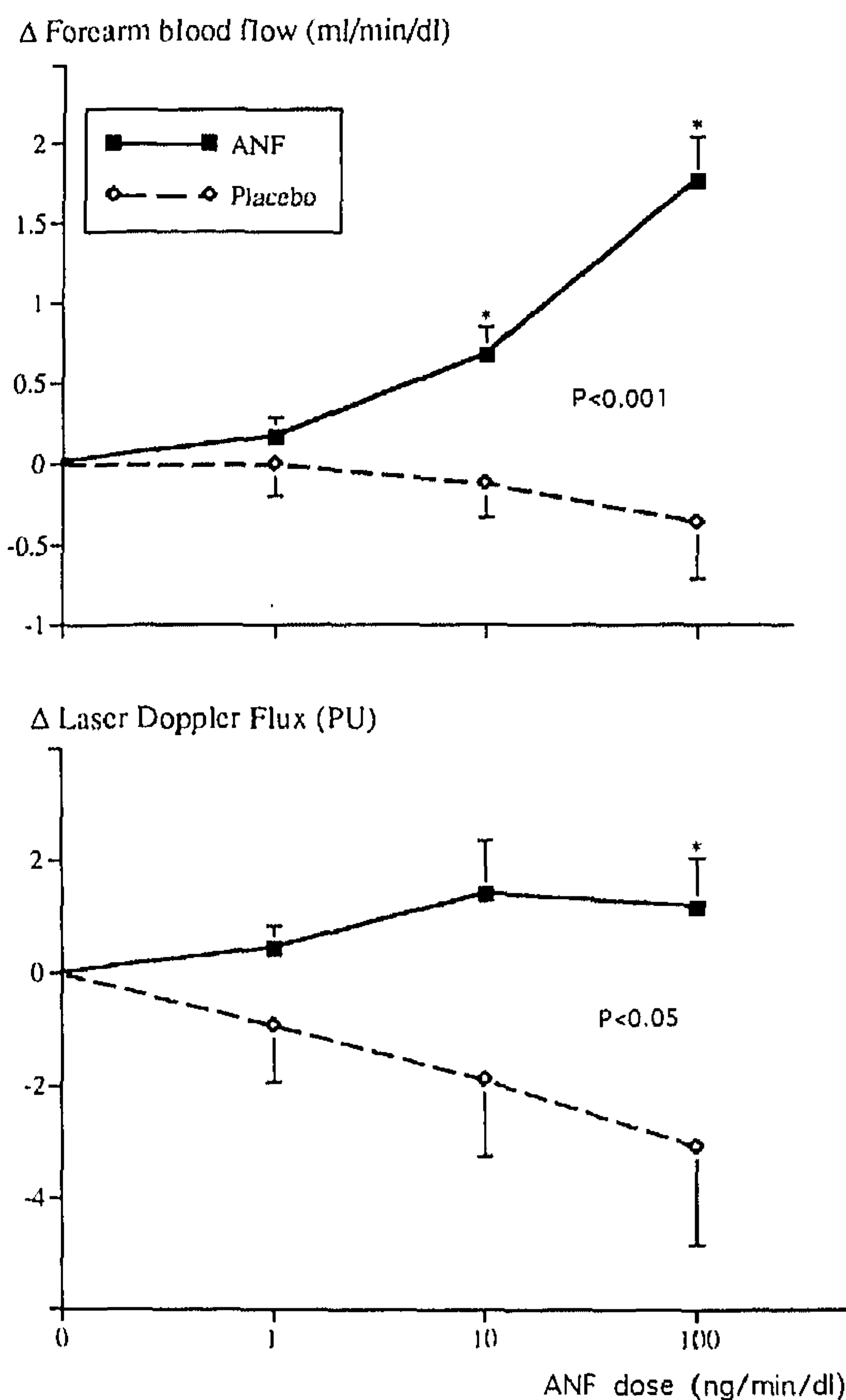


FIG. 1. Mean (\pm SE) changes from baseline of the forearm blood flow (Δ FBF) and the laser Doppler flux (Δ LDF) as induced by infusion of three increasing dosages of atrial natriuretic factor (ANF, solid squares/solid lines) or placebo (open diamonds/dashed lines). Indicated p-values refer to the comparison between ANF- and placebo-induced changes, assessed by repeated-measures analysis of variance. *Post hoc testing by Fisher protected least significant difference (PLSD) on placebo-corrected effect of ANF: $p < 0.05$ versus baseline.

± 0.4 ml/min/dl during the highest ANF dose ($p < 0.001$). Table 1 summarizes the percentage changes from baseline after ANF and after placebo and also shows a dose-dependent increase in FBF after ANF as compared with placebo.

The baseline LDF averaged 10.7 ± 2.6 PU in the placebo test and 8.5 ± 2.2 PU in the ANF test. This parameter increased slightly, though significantly, after the three ANF infusions (placebo-corrected

changes: 1.4 ± 1.3 , 3.3 ± 2.2 , and 4.2 ± 2.0 PU, $p < 0.05$). As shown in Table 1, the ANF-induced percentage changes from baseline were slight and not significantly different from those induced by placebo.

Effect of ANF on microvascular reactivity

In the placebo series, cold exposure to the fingers did not change the FBF (1.7 ± 0.1 at baseline and 1.6 ± 0.1 immediately after cold exposure, $n = 7$). However, during the procedure, LDF decreased from 9.9 ± 2.3 to 6.1 ± 2.0 PU. In the recovery phase, the LDF gradually increased to 7.0 ± 3.7 PU after 25 min. For the skin temperature, these values averaged $29.0^\circ \pm 0.5^\circ\text{C}$ at baseline, $21.2^\circ \pm 0.5^\circ\text{C}$ immediately after cold exposure, and $23.1^\circ \pm 1.6^\circ\text{C}$ after 25 min of recovery. Although the highest dose of ANF increased the baseline values of FBF and LDF, the course of these parameters after cold exposure was not changed by ANF as compared with placebo.

In the placebo series, the peak response of the LDF after occlusion of the finger was 21.1 ± 2.9 PU and reached 48.3 ± 13.4 PU seconds after the end of occlusion ($n = 7$). The LDF returned toward baseline level in 243.5 ± 36.3 s. In the presence of ANF, these values were comparable (peak response 21.6 ± 4.3 PU reached after 67.2 ± 18.0 s; recovery time 305.0 ± 62.3 s).

DISCUSSION

We demonstrated that infusion of ANF into the brachial artery induced a significant dose-dependent increase in FBF but only a slight increase in skin flux in healthy subjects. Plethysmographic FBF measurement includes both skeletal muscle BF and skin BF. The increase in FBF was more pronounced than the changes in skin flux. Therefore, the changes in the plethysmography values in this study must be attributed mainly to increases in skeletal muscle BF. The magnitude of the effects on skin microcirculation was remarkably small and appeared to occur only at the highest dose of ANF. We observed large differences between control values of LDF before ANF and placebo infusion. Because the sequence of testing was randomized, a time effect cannot explain this difference in baseline

TABLE 1. Mean (\pm SE) percentage changes from baseline for the Δ FBF and the Δ LDF during the three dosages of ANF and placebo

| Parameter | Placebo 1 | Placebo 2 | Placebo 3 | ANF-1 | ANF-2 | ANF-3 |
|------------------------------------|------------|------------|-------------|-----------|--------------|----------------|
| Δ FBF (%) ^a | 9 ± 10 | 5 ± 8 | -5 ± 9 | 4 ± 3 | 37 ± 9^b | 107 ± 22^b |
| Δ LDF (%) (NS) ^c | 1 ± 7 | 2 ± 10 | -6 ± 10 | 2 ± 5 | 11 ± 8 | 34 ± 21 |

FBF, forearm blood flow; LDF, laser Doppler flux; ANOVA, analysis of variance; ANF, atrial natriuretic factor.

^a $p = 0.0001$ repeated-measures ANOVA for ANF versus placebo.

^bPost hoc testing by Fisher PLSD on placebo-corrected effect of ANF: $p < 0.05$ versus baseline.

^cNo significant difference by repeated-measures ANOVA.

level. Large differences in LDF were observed and described previously. Despite this variability, LDF is considered useful in measuring skin microcirculatory reactivity during interventions (17,19). Previous studies in our department have shown that the regional ANF concentrations reached at the highest dose are much higher than in (patho)physiological conditions (20). By dividing the ANF infusion rate by the forearm plasma flow ($\text{FBF} \times [1 - \text{hematocrit}]$), the regional plasma ANF concentrations during the lowest dose of 1 ng/min/dl can be estimated as ~ 1 ng/ml, whereas physiological concentrations are generally < 0.1 ng/ml. Therefore, our results indicate that only suprphysiological levels of ANF induce a slight vasodilator response in the microcirculation.

Contradictory reports on the microcirculatory efficacy of ANF have been published previously (10,11). In a previous study, using the intravenous route of ANF administration, we reported that ANF was not a potent vasodilator in the skin microcirculation. However, systemic administration of ANF is complicated by cardiovascular reflex activity compensating for induced effects. Therefore, in the present study, we used the regional route of ANF administration to rule out the influence of compensatory mechanisms. Our current results suggest that ANF plays only a minor direct vasodilator role in normal skin microcirculation, in contrast to a more pronounced vasodilator effect on skeletal muscle BF.

We further investigated possible modulatory effects of ANF on the α -adrenoceptor-mediated microcirculatory constrictor response to finger cooling. The postsynaptic α -adrenoceptor-mediated vasoconstrictor response to cold exposure in human skin microcirculation is believed to be predominantly mediated by the α_2 -subtype (21). In vivo studies with selective α_1 - and α_2 -agonists have supported the concept of heterogeneity of postjunctional α -adrenoceptors in human vasculature. Faber and colleagues demonstrated that ANF was highly potent in selectively reversing α_1 -adrenoceptor-mediated constriction (13), but in their study α_2 -adrenoceptor-mediated constriction was ANF insensitive, which may explain the absence of an effect of ANF on cold-induced vasoconstriction in our study. Our experiments with postocclusive reactive hyperemia indicate that ANF apparently does not modulate microvascular vasodilator function.

ANF induces a direct dose-dependent vasodilator effect on the skeletal muscle vascular bed. In suprphysiological concentrations, ANF can also dilate the skin microcirculation, but does not appear to change microvascular reactivity.

REFERENCES

- Schwarz D, Geller DM, Manning PT, et al. Ser-Leu-Arg-Arg atriopeptin III: the major circulating form of atrial peptide. *Science* 1985;229:397-400.
- Burnett JC. Atrial natriuretic factor. Is it physiologically important? *Circulation* 1990;82:1523-4.
- Ballerman BJ, Brenner BM. Role of atrial peptides in body fluid homeostasis. *Circ Res* 1986;58:619-30.
- Johnston CI, Philips PA, Arnold L, Mooser V. Modulation of the renin-angiotensin system by atrial natriuretic peptide. *J Cardiovasc Pharmacol* 1990;16(suppl 7):S43-6.
- Thoren P, Mark AL, Morgan DA, et al. Activation of vagal depressor reflexes by atriopeptins inhibits renal sympathetic nerve activity. *Am J Physiol* 1986;251:H 1252-9.
- Winqvist RJ, Faison EP, Nutt RF. Vasodilator profile of synthetic atrial natriuretic factor. *Eur J Pharmacol* 1984;102:169-73.
- Osol G, Halpern W, Tesfamariam B, et al. Synthetic atrial natriuretic factor does not dilate resistance-sized arteries. *Hypertension* 1986;8:606-10.
- Koike H, Sada T, Miyamoto M, et al. Atrial natriuretic factor selectively increases renal blood flow in consciously hypertensive rats. *Eur J Pharmacol* 1984;104:391-2.
- Seymour AA, Blaine EH, Mazack EK, et al. Renal and systemic effects of synthetic atrial natriuretic factor. *Life Sci* 1985;39:33-44.
- Bussien JP, Biollaz J, Waeber B, et al. Dose-dependent effect of atrial natriuretic peptide on blood pressure, heart rate, and skin blood flux of normal volunteers. *J Cardiovasc Pharmacol* 1986;8:216-20.
- Webb DJ, Benjamin N, Allen MF, et al. Vascular responses to local atrial natriuretic peptide infusion in man. *Br J Clin Pharmacol* 1988;26:245-51.
- Jansen TLThA, Tan ACITL, Wollersheim H, et al. Age-dependent vasodilation of the skin microcirculation by atrial natriuretic factor. *J Cardiovasc Pharmacol* 1991;18:622-30.
- Faber JE, Gettes DR, Gianturco DP. Microvascular effects of atrial natriuretic factor interaction with α_1 - and α_2 -adrenoceptors. *Circ Res* 1988;63:415-28.
- Gamble J, Christ F, Gartside IB. Mercury in silastic strain gauge plethysmography for the clinical assessment of the microcirculation. *Postgrad Med J* 1992;68(suppl2):S25-33.
- Wollersheim H, Reyenga J, Thien Th. Laser Doppler velocimetry of fingertips during heat provocation in normals and in patients with Raynaud's phenomenon. *Scand J Clin Lab Invest* 1988;48:91-5.
- Marriott I, Marshall M, Johns EJ. Cutaneous vascular responses evoked in the hand by the cold pressor test and by mental arithmetic. *Clin Sci* 1990;79:43-50.
- Cleophas TJ, Fennis JF, van't Laar A. Finger temperature after a finger cooling test. Influence of air temperature and cigarette smoking. *J Appl Physiol* 1982;52:1167-71.
- Ninet J, Fronck A. Cutaneous postocclusive reactive hyperemia monitored by laser Doppler flow metering and skin temperature. *Microvasc Res* 1985;30:125-32.
- Bongard O, Fagrell B. Variations in laser Doppler flux and flow motion patterns in the dorsal skin of the human foot. *Microvasc Res* 1990;39:212-20.
- Jansen TLThA, Smits P, Tan ACITL, Thien Th. Attenuated forearm vasodilator response to atrial natriuretic factor in the elderly. *Hypertension* 1991;18:640-7.
- Coffman JD, Cohen RA. Role of adrenoceptor subtypes mediating sympathetic vasoconstriction in human digits. *Eur J Clin Invest* 1988;18:390-5.