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The albuminuric action of atrial natriuretic peptide is not modified by ACE-inhibition with perindopril in Type 2 diabetes

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Abstract

Aims Atrial natriuretic peptide (ANP) increases urine albumin excretion (UAER) in humans with Type 1 diabetes. The aim of this study was to establish if ANP increases UAER in microalbuminuric subjects with Type 2 diabetes and to examine whether the albuminuric action of ANP was inhibited by pre-treatment with the ACE-inhibitor perindopril.

Methods Seven microalbuminuric, normotensive males with Type 2 diabetes were entered into a randomised, double-blind, three-armed study of (i) intravenous infusion of ANP (0.25 μg/kg/min in 0.9% NaCl) after 3 weeks' pre-treatment with placebo, (ii) intravenous infusion of vehicle (0.9% NaCl only) after 3 weeks' pre-treatment with placebo, or (3) intravenous infusion of ANP (0.25 μg/kg/min in 0.9% NaCl) after 3 weeks' pre-treatment with perindopril, 4 mg daily.

Results Baseline parameters were similar on all three study days. During the placebo/vehicle arm there was no change in urine flow rate (UFR, $P = 0.61$), urine cyclic guanosine monophosphate (UcGMP $P = 0.48$) or UAER ($P = 0.99$). During the placebo/ANP arm there was a rise in UFR [13.7 ± 2.8 (mean ± SD) to 25.7 ± 7.7 mL/min, $P < 0.001$], UcGMP (60.0 ± 36.6 to 160.8 ± 118.5 μmol/mmolCr, $P = 0.045$) and UAER {5.13 [2.4–11.6] [median (range)] to 71.6 [21.6–175.1] mg/mmolCr, $P < 0.001$}. Pre-treatment with perindopril did not alter the changes in UFR ($P = 0.63$), UcGMP ($P = 0.46$) or UAER ($P = 0.99$) to infusion of ANP, compared with the placebo/ANP arm.

Conclusion ANP increases UAER in microalbuminuric patients with Type 2 diabetes and the albuminuric action of ANP is not inhibited by pre-treatment with the ACE inhibitor perindopril.

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Keywords atrial natriuretic peptide, diabetes mellitus, microalbuminuria

Abbreviations ANP, atrial natriuretic peptide; cGMP, cyclic guanosine monophosphate; GFR, glomerular filtration rate; UAER, urine albumin excretion rate

Introduction

Atrial natriuretic peptide (ANP) increases urine albumin excretion (UAER) in animals [1] and in humans with Type 1 diabetes [2–4]. Plasma levels of ANP are elevated in patients with Type 2 diabetes who have microalbuminuria, and also in conditions predisposing to the development of microalbuminuria [5–8], but it is not known whether there is a causal relationship between ANP and microalbuminuria in Type 2 diabetes.

ACE inhibitors reduce UAER and can prevent the development of microalbuminuria in normoalbuminuric normotensive patients with Type 2 diabetes [9]. We have shown that

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Table 1 Patient demographics, background diabetic retinopathy (BDR)

	Mean ± SD
Age (years)	50 ± 8.8
Duration (years)	7.1 ± 4.1
HbA_{1c} (%)	7.1 ± 1.7
SBP	138 ± 2.3
DBP	87.5 ± 3.5
UAER (mg/24 h)	76.5 ± 33.6
BMI (kg/m^2)	31.9 ± 6.1
Retinopathy status	4/7 had BDR on dilated fundoscopy

ACE inhibition prevents the albuminuric action of ANP in Type 1 diabetes [10], though other workers have disputed this effect [11]. It is possible that ACE inhibitors reduce albumin excretion rate by attenuation of the albuminuric response to ANP.

We examined whether intravenous infusion of ANP exerts an albuminuric effect in patients with microalbuminuria and Type 2 diabetes and whether pre-treatment with the ACE-inhibitor, perindopril could attenuate the albuminuric response to ANP.

Methods

Patients

Seven normotensive [blood pressure (BP) < 140/90 mmHg], microalbuminuric (30–300 mg/24 h) patients with Type 2 diabetes were recruited from the diabetes outpatient department (Table 1). Subjects with clinical evidence of macrovascular disease, cardiac, renal or hepatic failure were excluded. Approval was granted by the local ethics committee and all subjects gave written informed consent.

Methods

The design was a double-blind, randomised, placebo-controlled, three-armed crossover study, which comprised (i) a 60-min intravenous infusion of ANP (0.025 $\mu g\ kg^{-1}\ min^{-1}$) after 3 weeks' treatment with placebo (Servier, Paris, France), (ii) a 60-min isovolumetric infusion of vehicle (0.9% NaCl solution) after 3 weeks' treatment with placebo, (iii) a 60-min infusion of ANP (0.025 $\mu g\ kg^{-1}\ min^{-1}$) after 3 weeks' treatment with 4 mg perindopril (Servier) daily.

Patients presented at 08.00 h, fasting, having withheld their oral hypoglycaemics. Patients remained supine throughout the study, standing to urinate. Blood glucose was maintained between 4 and 6 mmol/l, using intravenous insulin (Actrapid, Novonordisk Copenhagen, Denmark). Once euglycaemic, patients were water loaded with 20 ml/kg tap water orally; urine was voided every 15 min and urine losses were replaced by equal volumes of water. Once steady state diuresis, was established, subjects received a 60-min infusion of either vehicle or ANP (Clinalfa, Läufel-Fingen, Switzerland), according to randomization.

Blood for ANP was collected into chilled tubes containing aprotonin (trasylol) and EDTA. After centrifugation at 4°C for 10 min, plasma was separated and stored at −80°C until assayed. Urine samples for cGMP (the secondary messenger for ANP) [12] and albumin were stored at −80°C until assayed. BP was measured by manual sphygmanometer. A 2-week washout period was allowed between each arm of the study.

Analysis

Urinary albumin, sodium and cGMP excretion were expressed as ratios to creatinine concentration, to correct for urine flow rate. Urine albumin was measured by enzyme-linked immunosorbent assay (Randox, Belfast, UK, limit of detection 0.01 mg/l, inter and intra-assay c.v. 3.9 and 5.2%, respectively). Urinary cGMP was measured by enzyme immunoassay (limit of detection 1 pmol/ml, interassay c.v. 9.7%). Plasma ANP was measured by immunoradiometric assay (Shinoria, Paris, France, limit of detection 2.5 pg/ml, inter and intra-assay c.v. 7.8 and 8.0%, respectively). Plasma renin activity was measured by radioimmunoassay (Diasorin, Saluggia, Italy).

Statistical analysis

Baseline parameters were compared using paired *t*-tests or the Wilcoxon test, as appropriate. Changes in parameters over time were analysed by one-way ANOVA and the difference between the two arms by two-way ANOVA with repeat measures using Microsoft Excel 7. The study had a 75% power to detect a change of 1 standard deviation and a 91% power to detect a change of 1.25 standard deviations.

Results

Baseline glucose, BP, urine flow rates (UFR), urine sodium excretion rate (UNaER), urine cGMP excretion rate (UcGMP) and UAER were similar on the three study days ($P > 0.1$ for all). Blood glucose, systolic and diastolic BP remained unchanged during all study arms (Fig. 1). Baseline PRA was higher in the perindropril/ANP arm than in the placebo/ANP arm [1.86 ± 0.98 (mean ± SD) vs. 0.67 ± 0.23 ng/ml/h, $P = 0.015$].

Baseline plasma ANP was lower in the placebo/vehicle arm than in the placebo/ANP arm ($P = 0.01$). Plasma ANP remained unchanged during the placebo/vehicle arm of the study [13.3 (2.3–17.3) median (range) to 14.4 (2.8–23.9) pg/mL, $P = 0.60$], but increased during the placebo/ANP arm [19.4 (4.5–31.7) to 310.3 (60.9–501.2) pg/mL, $P < 0.001$] ($P < 0.001$ vs. placebo/vehicle) and during the perindopril/ANP arm [10.3 (3.7–26.5) to 244.6 (42.0–625.4) pg/mL, $P < 0.001$]. There was no difference in plasma ANP after pre-treatment with perindopril compared with placebo ($P = 0.90$).

Patients maintained steady diuresis during the placebo/vehicle arm (11.0 ± 3.7 to 11.7 ± 5.1 ml/min, $P = 0.61$), but UFR increased during placebo/ANP arm (13.7 ± 2.8 to 25.7 ± 7.7 ml/min, $P < 0.001$) ($P = 0.004$ vs. placebo/vehicle) and the perindopril/ANP arm (13.4 ± 4.0 to 30.0 ± 6.0 ml/min, $P < 0.001$). The change in UFR in response to ANP was similar after pre-treatment with placebo or perindopril ($P = 0.63$) (Fig. 1).

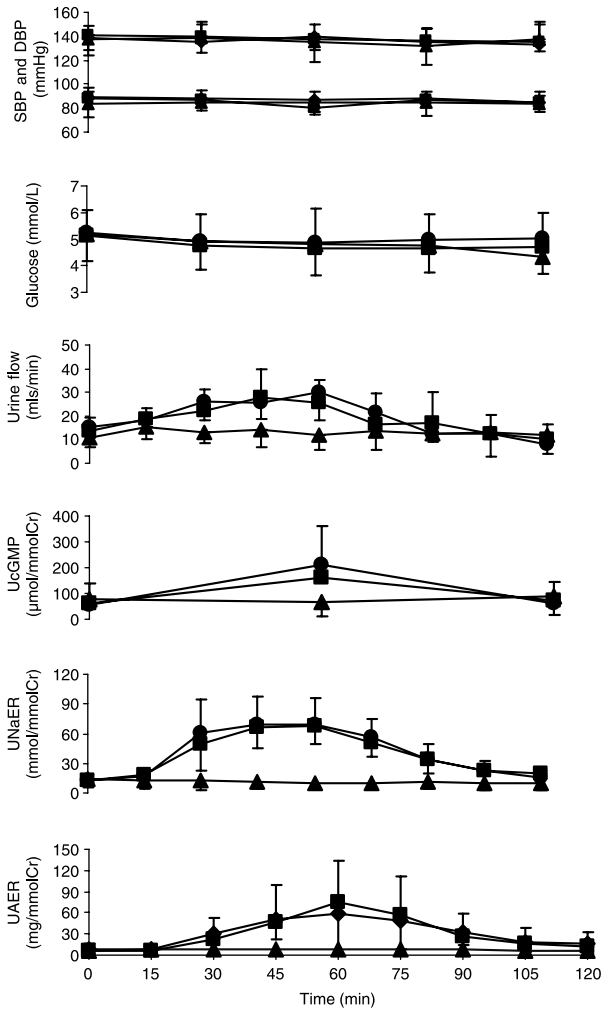

Figure 1 Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), blood glucose, urine sodium excretion rate, urine flow rate, urine cGMP and urine albumin excretion rate during placebo/vehicle arm (▲), placebo/ANP arm (■) and perindopril/ANP arm (●) in seven subjects with Type 2 diabetes mellitus. Results expressed as mean and standard deviation.

UNaER remained unchanged during the placebo/vehicle arm {14.6 [4.6–31.1] mmol/mmolCr [median (range)] to 11.8 [3.0–16.0] mmol/mmolCr, $P = 0.98$}, however, there was a marked natriuresis during the placebo/ANP arm [11.3 (5.0–10.2) to 73.5 (37.3–90.9) mmol/mmolCr, $P < 0.001$], ($P < 0.001$ vs. placebo/vehicle) and the perindopril/ANP arm [11.8 (5.3–23.5) to 84.7 (28.4–100.0) mmol/mmolCr, $P < 0.001$]. The natriuretic response to ANP was similar after pre-treatment with perindopril or placebo ($P = 0.98$) (Fig. 1).

UcGMP remained unchanged during the placebo/vehicle arm {63.3 [13.1–170.6] µmol/mmolCr [median (range)] to 46.1 [17.3–167.6] µmol/mmolCr, $P = 0.48$}, however, UcGMP increased during the placebo/ANP arm [52.9 (26.6–105.9) to 113.9 (63.7–415.5) µmol/mmolCr, $P = 0.045$]. The increase in UcGMP in response to placebo/ANP failed to achieve statistical significance when compared with placebo/vehicle ($P = 0.066$). UcGMP increased after the perindopril/ANP arm [59.7 (43.8–64.7) to 181.5 (44.4–503.1) µmol/mmolCr, $P = 0.01$]. There was no difference in the UcGMP response to ANP after 3 weeks' pre-treatment with placebo or perindopril ($P = 0.46$) (Fig. 1).

Urine albumin excretion remained unchanged during the placebo/vehicle arm {4.4 [2.9–16.4] mg/mmolCr [median (range)] to 5.1 [1.3–20.4] mg/mmolCr, $P = 0.99$}, however, there was an albuminuric response seen during the placebo/ANP arm [5.13 (2.4–11.6) to 71.6 (21.6–175.1) mg/mmolCr, $P < 0.001$]. The change in urine albumin excretion rate in response to placebo/ANP was significant when compared with placebo/vehicle ($P < 0.001$). After 3 weeks' treatment with perindopril, ANP still produced an increase in UAER [3.7 (0.5–22.3) to 38.9 (8.3–112.1) mg/mmolCr, $P = 0.03$]. There was no difference in the UAER response to 3 weeks' pre-treatment with placebo or perindopril ($P = 0.99$) (Fig. 1).

Discussion

We have demonstrated that intravenous infusion of ANP, producing plasma ANP concentrations in the pathophysiological range, increases urinary cGMP and UAER in patients with Type 2 diabetes and microalbuminuria. Furthermore, we have shown that pre-treatment with perindopril does not modify the natriuretic or albuminuric response to ANP.

ANP is elevated in Type 2 diabetes and microalbuminuria [5,6], and in conditions that predispose to the development of microalbuminuria [7,8]. This is the first study to confirm the albuminuric action of ANP in Type 2 diabetes and microalbuminuria. ANP has also been reported to increase UAER in normoalbuminuric Type 2 diabetes [13], and Type 1 diabetes [3,4]. This suggests that ANP may have a role to play in the development of microalbuminuria in Type 2 diabetes.

Plasma levels of ANP are increased in patients with cardiac disease such as myocardial infarction [14] or congestive cardiac failure [15], both conditions associated with increased UAER. In addition, it has been suggested that plasma ANP concentrations may be an index of cardiac dysfunction [16]. As there is good evidence that plasma ANP concentrations are elevated in conditions which predispose to microalbuminuria [7,8] and that plasma ANP concentrations are very closely related to the presence of microalbuminuria in Type 2 diabetes [5,6], the albuminuric action of ANP may be a causal link between cardiac dysfunction and microalbuminuria in diabetes.

The renal effects of ANP are mediated through changes within the glomerulus [1,3,17]. ACE inhibitors dilate the glomerular efferent arteriole, reduce intraglomerular hydraulic pressure, decrease filtration fraction and reduce basement membrane permeability [18,19], suggesting a potential for ACE inhibitors to antagonize the albuminuric actions of ANP. Although previous data has shown that quinapril blocks the albuminuric action of ANP in type 1 diabetes [10], our data, concurs with that of Zietse's group [11], which showed that enalapril did not effect the albuminuric action of ANP.

Perindopril (or perindoprilat, the active metabolite of perindopril) has similar lipid solubility [20,21] and bradykinin production [22–24] to quinapril, making it unlikely that intrinsic ACE inhibitor qualities account for the disagreement between trials. The profound effects seen in McKenna's study, which employed a 1-week pre-treatment with quinapril to block the albuminuric action of ANP may reflect acute haemodynamic changes immediately after ACE inhibition [25]; both our study and that of Zietse had longer pre-treatment periods and acute haemodynamic effects may have worn off, whereas the more chronic effects of ACE inhibitors to reduce albuminuria, which depend on changes in basement membrane permeability, and which vary according to ACE-Inhibitor may not yet have occurred [26].

We have shown that intravenous infusion of ANP increases UAER in microalbuminuric Type 2 diabetes, supporting the theory that elevated levels of ANP as reported in this population may have a role in the progression of microalbuminuria to nephropathy in Type 2 diabetes. Pre-treatment with the ACE inhibitor perindopril failed to block the albuminuric actions of ANP.

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