Plasma atrial natriuretic polypeptide concentrations during and after reversion of paroxysmal supraventricular tachycardias

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SUMMARY Plasma concentrations of immunoreactive atrial natriuretic polypeptide were raised in 22 of 23 patients with paroxysmal supraventricular tachycardia and in all seven patients with atrial flutter. Plasma concentrations of atrial natriuretic polypeptide rose soon after the onset of supraventricular tachycardia. A sample taken 30 minutes after reversion to sinus rhythm (pharmacological or non-pharmacological) showed a significant fall in 19 of the 23 patients with paroxysmal supraventricular tachycardia and all seven patients with atrial flutter.

Because atrial natriuretic polypeptide has powerful natriuretic and diuretic properties, an increase may contribute considerably to the polyuria that is often associated with episodes of supraventricular tachycardia.

Mammalian atrial tissue has been shown to contain secretory-like storage granules with characteristics similar to those of endocrine peptide secretory cells.^{1,2} These granules were shown to contain a polypeptide hormone, which is first synthesised as a 151 amino acid prohormone.^{3,4} The prohormone is further processed to several smaller peptides, one of which is α natriuretic polypeptide, a 28 amino acid polypeptide with potent natriuretic, diuretic, and vasorelaxant properties.⁵⁻⁷

The physiological role of atrial natriuretic polypeptide, or indeed any of the post-translational products, is unknown; however, there is a considerable body of evidence to suggest that this peptide may help to maintain sodium ion and water balance and modulate the renin-angiotensin aldosterone system.⁸ A major stimulus for the release of atrial natriuretic polypeptide into blood seems to be atrial distension produced by volume loading or increased dietary intake of sodium ion.^{9,10}

Paroxysmal supraventricular tachycardias are often associated with considerable polyuria and reports of raised concentrations of plasma atrial natriuretic polypeptide in such patients suggest that

this substance may account for the combined diuresis and natriuresis.¹¹⁻¹³

We measured plasma concentrations of atrial natriuretic polypeptide before and after reversion to sinus rhythm in patients with paroxysmal supraventricular tachycardia and those with atrial fibrillation or flutter.

Patients and methods

PATIENTS

This study was carried out within the guidelines set down by the National Health and Medical Research Council of Australia and was approved by the institute's human ethics advisory committee.

We studied twenty three patients admitted to our casualty department with paroxysmal supraventricular tachycardia (18 women and 5 men, aged 17-81 (mean 53) years). Diagnosis was confirmed by 12 lead electrocardiography which showed a regular, narrow QRS tachycardia in all 23 patients. Patients with acute or chronic congestive cardiac failure were specifically excluded.

In addition, we studied seven patients (five men and two women (mean age 60) with atrial fibrillation or flutter and five control patients (three men and two women (mean age 50)) without arrhythmias who had had intravenous lines inserted in the casualty department for other indications.

Renal function was assessed in all patients. Plasma

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creatinine concentrations were within the normal range (0.06–0.12 mmol/l) in all but two patients (0.14 and 0.21 mmol/l).

Fifteen patients were receiving concomitant medication when they were studied. This included a β blocker in one, β blocker plus digoxin in two, digoxin alone in one, quinidine in two, verapamil in one, diuretic agents in three, theophylline in one, thyroxine in two, and non-steroidal anti-inflammatory drugs in two.

A first blood sample was taken after insertion of a peripheral intravenous line and before attempted reversion and a second sample was taken 30 minutes after reversion to sinus rhythm.

BLOOD COLLECTION AND RADIOIMMUNOASSAY OF ATRIAL NATRIURETIC POLYPEPTIDE IN PLASMA

Blood collection and extraction

Venous blood (10 ml) was collected into a chilled tube containing edetic acid and aprotinin (10 000 units; Bayer, Australia). We took care to avoid haemolysis, which causes loss of immunoreactive atrial natriuretic polypeptide in plasma. Plasma was separated immediately by centrifugation, snap frozen, and stored at -20°C until assay.

Extraction of plasma

Plasma (1 ml) was extracted overnight at 4°C with 1.8 ml acidified ethanol (95: 5 parts of 95% ethanol: 6 mol/l acetic acid) followed by centrifugation, and lyophilisation of the supernatant. The dried extract, equivalent to 1.0 ml of plasma, was reconstituted with 0.50 ml of assay buffer (radioimmunoassay section) and assayed for atrial natriuretic polypeptide.

The efficiency of the extraction procedure was determined for each patient's sample by the addition of radiolabelled human α atrial natriuretic polypeptide to a separate 1 ml volume of patient's plasma. This plasma was extracted as described above. After reconstitution in assay buffer (0.50 ml) a 200 μl volume was taken and its radioactive content measured and recovery was estimated. Recovery of radiolabelled human α atrial natriuretic polypeptide from plasma samples from both the controls and the patients was 78(6)% (mean (SEM)).

Radioimmunoassay of atrial natriuretic polypeptide

Assay buffer.—For all procedures in the assay we used 0.05 mol/l sodium phosphate buffered saline pH 7.4 containing aprotinin (250 U/ml), edetic acid (0.01 mol/l), 0.25 g % bovine serum albumin (Sigma Chemical, radioimmunoassay grade), and thiomersal (0.6 mmol) (Sigma Chemical, as a preservative).

Specificity and characterisation of antiserum.—Antiserum to atrial natriuretic polypeptide 1–28 was raised in sheep after conjugation of synthetic human α atrial natriuretic polypeptide 1–28 to bovine thyroglobulin (Sigma Chemical USA) with ethylcarbodiimide (Pierce Chemicals, USA). The antiserum cross reacted to an equal degree with atriopeptins I, II, III, but it did not cross react with cardiodilatin 1–16, angiotensin II, vasopressin, oxytocin, neuropeptide Y, somatostatin, β endorphin, leu and met enkephalins and dynorphins 1–8 and 1–13.

We used the MK model extended least squares non-linear curve fitting program to analyse antibody binding affinity. Two binding sites were found: the first had a dissociation constant (K_d) of 37.58 pmol/l and affinity constant (K_a) of 2.7×10^7 l/pmol and the second a K_d of 122.5 pmol/l and K_a of 0.08×10^7 l/ μmol .

Radioimmunoassay was carried out in 10×75 mm borosilicate glass tubes under non-equilibrium conditions at 4°C . Duplicate volumes (0.20 ml) of the plasma extract were incubated with 100 μl of sheep antiserum to human α atrial natriuretic polypeptide (final concentration 1:6000), followed by a further two day incubation, after the addition of 100 μl of (3-[^{125}I] iodotyrosyl 28) α atrial natriuretic polypeptide (5000 counts/min equivalent to 1–2 pg tracer mass) (Amersham, Australia). Synthetic human α atrial natriuretic polypeptide was used to generate a standard curve over the range 2–1000 pg/tube.

Bound and free atrial natriuretic polypeptide were separated by the addition of 100 μl of carrier sheep serum (diluted 1:250) and 100 μl of donkey antisheep immunoglobulin serum (diluted 1:5) followed by incubation at room temperature for three hours and centrifugation. The pellet was counted in a NEN 1600 gamma counter (Nuclear Enterprises). Standards were set up in triplicate in the presence of 0.20 ml of an extract of charcoal stripped plasma (hormone free) so as to reproduce the assay conditions used for the patient plasma samples. Concentration of plasma atrial natriuretic polypeptide was determined from the standard curve, plotted as the logit-log of the ratio of bound counts of unknown to bound counts of the zero standard versus concentration (pg/tube). The standard curve was linear over the range 10 to 600 pg/tube and the sensitivity of the assay was 2 pg/tube (2 from the mean $\pm$ SD of the zero standard). The intra and inter assay variability were 8 and 15% respectively.

Characterisation of plasma atrial natriuretic polypeptide measured by radioimmunoassay.—Plasma samples collected before and after reversion were pooled separately, extracted as above, and the dried

extracts were analysed by high pressure liquid chromatography on a C18 reverse phase column and with an acetonitrile linear gradient elution profile. Analysis of the column eluant showed one major peak of immunoreactivity for both the plasma samples that coincided with the elution profile of the synthetic human α atrial natriuretic polypeptide (results not shown).

The plasma extracts were further characterised by serial dilution experiments. Pooled extracts of plasma obtained before and after reversion diluted in parallel with the synthetic human α atrial natriuretic polypeptide standard (results not shown).

Concentration of atrial natriuretic polypeptide in controls.—Atrial natriuretic polypeptide was measured in blood samples from 34 normotensive healthy volunteers (14 men, 20 women) with normal dietary intake of sodium and electrolyte status which were treated and extracted as described above. Subjects remained seated for 15 minutes before a blood sample was taken between 9 am and 10.30 am. The upper and lower limits of the normal range were 70 pg/ml and 4 pg/ml respectively and no sex dependent differences were found (men 36 (14) pg/ml, women 33 (16) pg/ml) (mean (SEM)).

Fig 1 Plasma concentration (mean (SEM)) of atrial natriuretic polypeptide (ANP) (pg/ml) before and 30 minutes after reversion in 23 patients with paroxysmal supraventricular tachycardia. The normal range is also shown.

Results

Figure 1 shows plasma concentrations of atrial natriuretic polypeptide before and after reversion to sinus rhythm in the 23 patients with paroxysmal supraventricular tachycardia. During paroxysmal supraventricular tachycardia 22 of 23 patients had atrial natriuretic polypeptide concentrations above the upper limit for normal subjects in this laboratory (70 pg/ml). In one patient plasma atrial natriuretic polypeptide was within the normal range and did not change after reversion. Paroxysmal supraventricular tachycardia was converted to sinus rhythm by intravenous verapamil (1–20 mg) in 16 patients, intravenous digoxin (250–500 μ g) in one, vagal manoeuvres in three, or a combination of these in three. In 19 patients concentrations of plasma atrial natriuretic polypeptide fell 30 minutes after reversion, in five to within the normal range. The mean concentration of plasma atrial natriuretic polypeptide was 256 (43) pg/ml before reversion and 120 (19) pg/ml 30 minutes after reversion ($2p < 0.005$ by paired Student's t test). The fall in the concentration of atrial natriuretic polypeptide seemed to be independent of the mode of reversion of paroxysmal supraventricular tachycardia.

There was a weak inverse relation between the rate of paroxysmal supraventricular tachycardia (140–220 beats/min) and plasma concentrations of atrial natriuretic polypeptide ($r = -0.45$, $p = 0.017$ by

Fig 2 Plasma concentration (mean (SEM)) of atrial natriuretic polypeptide (ANP) (pg/ml) before and 30 minutes after reversion in seven patients with atrial fibrillation or flutter. The normal range is also shown.

Pearson's test). The duration of paroxysmal supraventricular tachycardia (10 min–3 days) had no apparent effect on plasma atrial natriuretic polypeptide concentrations; one patient had considerably raised plasma atrial natriuretic polypeptide (84 pg/ml) with paroxysmal supraventricular tachycardia lasting only 10 minutes.

In the seven patients with atrial fibrillation or flutter, plasma concentrations of atrial natriuretic polypeptide were raised before reversion was attempted (156 (33) pg/ml) and fell after reversion (68 (22) pg/ml) (fig 2).

Discussion

Our results show that patients with supraventricular tachycardia, particularly paroxysmal supraventricular tachycardia, and atrial fibrillation or flutter have considerably higher plasma concentrations of atrial natriuretic polypeptide than healthy controls. These results are consistent with earlier studies of small numbers of patients and animal studies.^{13–19} Given that atrial natriuretic polypeptide has natriuretic and diuretic properties, the increase may account for the polyuria reported in patients with supraventricular tachycardias.^{12 20 21} Others have suggested that the polyuria may be caused by suppressed vasopressin release^{21 22}; however, such a mechanism does not account for the striking natriuresis associated with this condition.²⁰

Plasma concentrations of atrial natriuretic polypeptide may rise early after the onset of paroxysmal supraventricular tachycardia and remain raised for prolonged periods of tachycardia, independent of clinical indices of left ventricular failure. The weak inverse relation between the rate of paroxysmal supraventricular tachycardia and plasma concentrations of atrial natriuretic polypeptide remains unexplained. One possible explanation is that the atrial cardiocyte becomes fatigued and supplies of atrial natriuretic polypeptide are exhausted. This would be an unusual phenomenon in endocrine cells but would be consistent with the fall in concentrations of atrial natriuretic polypeptide during tachycardia in one of the patients with atrial flutter (119 pg/ml to 44 pg/ml) that occurred without a change in rate of tachycardia and before reversion was attempted.

Although atrial pressure increases in this type of tachycardia and may act as the stimulus for secretion of atrial natriuretic polypeptide,¹¹ alternative intrinsic mechanism(s) associated with the arrhythmias could also mediate release of atrial natriuretic polypeptide. Further studies will be required to elucidate the mechanism(s) responsible.

In conclusion, supraventricular tachycardia (and

paroxysmal supraventricular tachycardia in particular) are associated with raised concentrations of plasma atrial natriuretic polypeptide that fall rapidly and apparently independently of the mode of reversion whether this is pharmacological or non-pharmacological.

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