Urinary cyclic adenosine monophosphate in young adults and elderly subjects¹

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SYNOPSIS The 24-hour urinary excretion of cyclic 3',5'-adenosine monophosphate (cAMP) was measured by a protein-binding assay in 55 healthy volunteers (aged 20-35 yr) and in 30 hospitalized elderly subjects (aged 70-93 yr). In the older subjects the mean 24-hour cAMP excretion was significantly lower; the correlation between cAMP excretion and age demonstrated a progressive decrease from the age of 70 to the tenth decade. Many different factors could account for the reduced urinary cAMP excretion in elderly subjects: a decline in the reactivity of the adenyl cyclase-cAMP system related to physiological ageing; reduced physical activity; a reduction in the glomerular filtration rate or decreased production of cAMP by tubular cells in the senile kidney.

Following the demonstration that cyclic 3',5'-adenosine monophosphate (cAMP) appears in urine (Butcher and Sutherland, 1962), several investigators sought to explain its origin and significance. Exercise (Eccleston *et al*, 1970), upright posture (Hamet *et al*, 1973), pregnancy (Taylor *et al*, 1970), glucagon (Broadus *et al*, 1970b), vasopressin (Takahashi *et al*, 1966; Chase and Aurbach, 1967), catecholamines (Ball *et al*, 1972), and parathyroid hormone (Chase and Aurbach, 1967; Kaminsky *et al*, 1970; Taylor *et al*, 1970) influence the urinary excretion of cAMP. Support for the kidney as the tissue of origin of the increased urinary cAMP excretion has come only from studies on the renal action of parathyroid hormone and vasopressin (Chase and Aurbach, 1968). The demonstration of increased urinary cAMP excretion in hyperparathyroid patients led to the investigation of the clinical usefulness of this test in the differential diagnosis of hypercalcaemic states (Murad and Pak, 1972; Neelon *et al*, 1973; Mallette *et al*, 1974). As it concerns normal subjects, the 24-hour urinary excretion of cAMP has been evaluated in adults and children (Linarelli, 1972). Apart from these observations, we know of no systematic study of the effect of age on the urinary excretion of cAMP. We report here 24-hour urinary cAMP data for men and women from the third to the tenth decades of life.

Methods

The 24-hour urinary excretion of cAMP was determined in 55 healthy volunteers aged 20-35 years, mostly university students, who were unrestricted in regard to diet and activity, and in 30 hospitalized subjects aged 70-93 years without symptoms or history of peptic-ulcer disease, hypercalcaemia, bone disease, renal stones, renal insufficiency, essential hypertension or diabetes.

Twenty-four-hour urines beginning at 0800 were collected under refrigeration. At the end of the urine collection periods aliquots of urine were frozen at -20°C until analysed for cAMP and creatinine content. Fifteen of the younger subjects had sequential 24-hour urines collected on several days.

A 50 µl aliquot from each sample was assayed by the 'cyclic AMP assay kit' of the Radiochemical Centre, Amersham, UK. The cyclic AMP assay is based on the competition between unlabelled cyclic AMP and a fixed quantity of the tritium-labelled compound for binding to a protein which has a high specificity and affinity for cyclic AMP; the method has been described in detail by Tovey *et al* (1974).

Results

The mean 24-hour urinary cAMP excretions in young adults and elderly subjects are shown in fig 1. Normal young adults excreted 3.61 ± 0.19 SEM µmol daily; by contrast, in elderly subjects the mean 24-hour cAMP excretion was significantly

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Fig 1 Mean ($\pm$ SEM) 24-hour urinary cAMP excretion (left) and cAMP-creatinine ratio (right) in a group of 55 normal adults (20-35 yr) and in 30 elderly subjects (70-93 yr).

Fig 2 Plot of cAMP excretion ($\mu\text{mol/day}$) against creatinine excretion (g/day) in 55 normal young adults (20-35 yr) and in 30 elderly subjects (70-93 yr).

lower: 1.78 ± 0.12 SEM μmol daily ($p < 0.001$). Expressing these levels in relation to the quantity of creatinine that was concomitantly excreted, the difference between the two groups was reduced: in young subjects the mean cAMP-creatinine ratio ($\mu\text{mol/g}$) was 3.73 ± 0.20 SEM in older subjects 2.79 ± 0.10 SEM ($p < 0.01$). A plot of the cAMP excretion per 24 hours against excretion per gram creatinine (fig 2) showed a highly significant correlation in all subjects tested (regression line $y = 1.23 \pm 1.81x$, $r = 0.59$, $p < 0.001$). The observed relationship between cAMP excretion and creatinine excretion confirms a previous report in normal subjects (Kaminsky *et al*, 1970). In the group of subjects aged 20-35 years the correlation between cAMP excretion and age was not significant ($p > 0.5$); on the contrary, the correlation between age and 24-hour urinary cAMP excretion in older subjects, as illustrated in fig 3, was highly significant (regression line $y = 7.175 - 0.068x$, $r = 0.66$, $p < 0.001$). The

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regression slope demonstrated a progressive decrease in cAMP excretion from the age of 70 to the tenth decade.

There was a positive correlation ($r = 0.61$, $p < 0.001$) between cAMP excretion and body surface area (fig 4), according to the data previously reported in normal subjects (Taylor *et al*, 1970). No

Fig 3 Relationship between urinary cAMP excretion ($\mu\text{mol/day}$) and age in 30 older subjects. In the group of 55 younger subjects the correlation was not significant ($p > 0.5$).

Fig 4 Plot of cAMP excretion ($\mu\text{mol/day}$) against the body surface area (m^2) in 55 normal young adults (20-35 yr) and in 30 elderly subjects (70-93 yr).

significant differences in cAMP excretion in men and women were observed. Only small variations in urinary cAMP excretion from day to day were found in individual subjects.

Discussion

There seems, therefore, to be no doubt that urinary cAMP excretion falls with age in older people of both sexes. We cannot be certain what causes this decrease: cAMP in urine comes both from plasma by glomerular filtration and from renal tubular cells by leakage into the tubular fluid. Under normal conditions the urinary excretion of cAMP is largely a function of glomerular filtration (Broadus *et al*, 1970a), so that in normal subjects cAMP excretion is significantly correlated with creatinine excretion (Kaminsky *et al*, 1970); the nucleotide production in plasma may well be the result of small net contributions from many tissues. The remaining aliquot of the urinary nucleotide represents nephrogenously generated cAMP, and this nephrogenous contribution is for the most part under the control of parathyroid hormone.

Therefore, several possible mechanisms could account for the reduced urinary cAMP excretion in elderly subjects.

First, it might reflect a general decline with age in the reactivity of adenyl cyclase in response to hormonal stimulation at various cell membrane levels. Little information is available concerning the major sources (liver, kidney, and adrenals) of plasma cAMP under basal conditions. The possibility that other regions, such as adipose tissue and skeletal muscle, may contribute markedly to the circulating levels of the nucleotide cannot be excluded (Wehmann *et al*, 1974). A close correlation between lean body mass and urinary creatinine has previously been shown (Miller and Blyth, 1952): thus, in elderly subjects, the reduced lean body mass could be related to a decreased excretion of both cAMP and creatinine.

Secondly, the 24-hour excretion of cAMP was found to increase with exercise (Eccleston *et al*, 1970): a lack of activity may be one of the factors leading to the low values obtained in our older hospitalized subjects when compared to those in younger persons.

Thirdly, in normal old age nephrons become obsolescent and the glomerular filtration rate falls (Davies and Shock, 1950): the reduction in glomerular filtration rate might contribute to a decrease in the filtered load of the nucleotide. In addition, the low urinary excretion of cAMP might reflect a decline in the activity of the adenyl cyclase-cAMP system in the senile kidney. About one-third of the

cAMP excreted in the urine is produced by tubular cells (Kaminsky *et al*, 1970).

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