Presence of atrial natriuretic factor and cyclic guanosine monophosphate in saliva. Comparison of plasma and salivary concentrations during a head-down tilt

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Summary. Using a specific and sensitive radio-immunoassay involving separation and extraction procedures, we measured the concentration of saliva and plasma atrial natriuretic factor (ANF) and cyclic guanosine monophosphate (cGMP) in men before and during a 10 h head-down tilt at -6°. Saliva values closely correlated with plasma for ANF (r=0.7–0.95) and for cGMP (r=0.65–1). During this dynamic test, the mean concentrations of ANF and cGMP were significantly higher after 15 and 45 min, respectively, this increase persisting for 3.5 h. We concluded that the concentration of ANF in saliva may be significantly affected by a marked fluid shift from the lower to the upper half of the body. This is the first time that the presence of ANF and cGMP has been demonstrated in saliva. The great advantage of studying saliva is that it can be obtained non-invasively in athletes or during space flight. This methodology will be used during the Soviet-French space flight (Antares Project) planned for 1992.

Key words: Atrial natriuretic factor – Cyclic guanosine monophosphate – Saliva – Head-down tilt

Introduction

Many studies on the early cardiovascular and hormonal changes induced by space flight or simulated weightlessness have been published (Leach et al. 1988, 1989; Gharib et al. 1988; Greenleaf 1984). It is generally admitted that the initial changes induced by space flight or head-down tilt (HDT) as well as ground-based studies are characterized by a reduction in hydrostatic gradient leading to a headwards shift of fluid with increased central venous pressure, which is the starting point of reflexes triggered by the arterial baroreceptors, influencing both heart rate and peripheral resistance of the arterial bed. Increase in atrial pressure is a well-known and important stimulus for the release of atrial natriuretic factor (ANF) from atrial cardiomyocytes (Ruskoaho et al. 1987). The physiological signal given by release of ANF into the blood has been shown to be transmitted intracellularly by cyclic guanosine monophosphate (cGMP; Hamet et al. 1984; Waldman et al. 1984). Also, a high concentration of ANF in plasma in vivo has been found to cause an increase in cGMP concentrations in plasma (Gerzer et al. 1985). Two iso-enzymes of guanylate cyclase have been suggested to be responsible for cGMP production (Leitman et al. 1987): a soluble form, which can be stimulated by organic nitrates such as glycerol trinitrate or sodium nitroprusside (Wood et al. 1987), and a particulate form, which represents the intracellular domain of a transmembrane protein carrying an ANF receptor on the external surface of the membrane.

However, in space physiology, it is difficult to obtain blood from astronauts during flight. This has also been the case in respect of athletes during exercise. Thus, we decided to measure ANF in saliva. Saliva analysis offers many advantages: stress can cause the elevation of many hormones and saliva sampling can avoid the pain and apprehension sometimes associated with venipuncture. Dynamic tests of endocrine function often require multiple samples; and saliva sampling avoids the need for repeated venipuncture. The easy, stress-free, noninvasive collection procedure can greatly facilitate the study of normal subjects or patients (Ben-Aryeh et al. 1989; Cook et al. 1987).

Our aim was to:
1. Demonstrate the presence of ANF and cGMP in saliva
2. Examine the relationship between concentrations in serum and saliva during the first hours of a HDT at -6°, a situation which is now well-known to increase the release of ANF (Gharib et al. 1987).

Methods

Subjects and test design. A group of 5 healthy male volunteers (G, J, L, P, Q) aged 30–40 years, participated in this study. The de-
Fig. 1. Changes in plasma and saliva concentrations (mean and SEM) of atrial natriuretic factor (ANF) and cyclic guanosine monophosphate (cGMP) during a head-down tilt (HDT) at −6° (C = control period)

tails of the protocol were explained to each subject and their informed consent was obtained. The protocol had be approval of the National Ethics Committee. On the experimental day, a catheter was inserted into an antecubital vein 1 h before the first blood sampling. Room temperature was maintained at 20–21°C. Blood and saliva samples were taken 5 min before, and 15 min, 45 min, 75 min, 105 min, 2.5 h, 3.5 h, 5 h, 9 h and 10 h after HDT at −6°.

To establish reference values for cGMP, we took blood and saliva samples from 15 healthy volunteers who were not receiving any drug therapy.

Collection of samples. Blood was collected by venipuncture from an antecubital vein into heparinized tubes. The separated serum was frozen at −20°C until assayed. Unstimulated whole saliva was collected with Salivette (Sarstedt, Numbercht, FRG). Briefly, the cotton was placed under the tongue, the sample taken then centrifuged and frozen. The generally applied technique of saliva preparation before assay is freezing, thawing and centrifugation, which produces a clear, easily pipettable supernatant. Freezing and thawing of saliva, however, results in precipitation of globular proteins; centrifugation leads to a loss of only protein content (Vining et al. 1983; Vining and McGinley 1986; Schramm and Smith 1991; Meulenberg and Hofman 1990).

Sample preparation. The saliva samples were thawed at room temperature. After thorough mixing, each sample was centrifuged for 10 min at 1500g, which yielded a clear saliva supernatant. Molecules less than 8000 Da were excluded with membrane cellulose ester (Centri/Por, Spectrum, Los Angeles, Calif.), so that potential blood contamination in saliva did not interfere with the analysis.

Assay procedure for determination of ANF. A 1-ml sample of saliva and plasma was extracted by C18 Sep-Pak cartridges (Waters...