Atrial Natriuretic Peptide (ANP) Attenuates Brain Oedema Accompanying Experimental Subarachnoid Haemorrhage*

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Summary

The effects of centrally administered atrial natriuretic peptide (ANP) on the brain water and electrolyte contents were investigated in a rodent subarachnoid haemorrhage (SAH) model. SAH caused statistically significant increases in the brain sodium and water contents, while the potassium content did not change significantly, indicating that the brain oedema could be classified as having a primarily vasogenic component. Two μg or 5 μg of rat ANP administered into the lateral ventricle at the time of SAH induction statistically significantly decreased the water and sodium accumulation measured 90 minutes following SAH. The same treatment did not inhibit development of brain oedema measured 3 hours following SAH. However, when 5 μg of ANP was administered intraventricularly at the time of SAH induction and also 90 minutes later, the brain oedema 3 hours following SAH was again reduced statistically significantly. These effects of ANP were found not to be mediated by primary changes in serum osmolality and electrolyte concentrations.

The present results confirm that centrally administered ANP may act directly on the central nervous system to inhibit brain water and sodium accumulation in SAH-induced brain oedema. The potentials of influencing the central neuro-endocrine system as a novel way of the treatment of brain oedema are discussed.

Keywords: Subarachnoid haemorrhage; SAH; natriuretic peptide; ANP; brain oedema.

Introduction

Aneurysmal subarachnoid haemorrhage (SAH) is known to result in both generalized and focal disturbances of the brain function⁴⁶. A combination of different factors of similar pathogenic importance is thought to lead eventually to these alterations. First, the normal brain metabolism is disrupted by direct exposure of the brain tissue to blood²⁵, ⁴². Second, the raised intracranial pressure (ICP) resulting from the sudden entry of blood into the subarachnoid space may injure the brain²⁰, ⁴⁶. Third, the nervous tissue may be rendered ischaemic by the reduced arterial perfusion pressure and spasms of the cerebrovasculature which frequently accompany SAH¹¹, ²¹, ⁴⁶. Fourthly, it has been shown that the increased permeability of brain microvessels caused by a subarachnoid blood clot may lead to the development of vasogenic oedema (disruption of the blood-brain barrier/BBB) in addition to cytotoxic (ischaemic) brain swelling¹¹, ³⁷, ⁴⁷. The aim of neurosurgical treatment is to prevent secondary brain damage and rebleeding.

Many of the hormones involved in the regulation of the systemic fluid and electrolyte metabolism are synthesized and released within the central nervous system (CNS)³⁸, ⁴⁰. These are atrial natriuretic peptide (ANP), vasopressin (AVP), angiotensin and endogenous digoxin¹⁵, ⁴⁹. A considerable amount of evidence indicates that the brain is capable of controlling its fluid and electrolyte balances under a variety of circumstances³⁸. Various cell groups, such as the brain capillary endothelial cells and the secretory cells of the choroid plexus and astroglia, are responsible for precise control of the brain and intracranial volumes. Raichle in 1981, and Dőczi et al. 1982, put forward the hypothesis that a central neuro-endocrine system regulates the brain bulk and ion content⁹, ³⁸. The hypothesis proposes that three cell groups control the internal ionic environment of the brain. A unique element of the hypothesis is that this regulation is orchestrated by a central neuro-endocrine system capable of affecting all
three cell types. Centrally released neuropeptides may act locally on adjacent target cells or on more distant intracerebral sites following distribution through the brain extracellular fluid (ECF) and/or cerebrospinal fluid (CSF), i.e. they may act within the CNS either as transmitters or as hormones. AVP and ANP probably function within the brain via a paracrine mechanism, as physiological regulators of brain cell and interstitial fluid (ISF) volume. AVP and ANP are released in the CNS independently from the periphery, and influence tissue water conservation (AVP) and depletion (AVP) directly. Accordingly, it is important to investigate the effects of ANP on the brain water and electrolyte contents in SAH, where the balance of these constituents is disturbed and may lead to secondary brain damage.

The aim of the present investigation was to study the effects of intraventricularly administered ANP on the brain water, sodium and potassium contents in a rat SAH model described and characterized earlier. The possible mechanism for the effects of ANP on brain oedema following SAH is also discussed.

**Material and Methods**

**Experimental Design: Induction of SAH**

The experiments were performed on adult Wistar rats of both sexes, ranging in weight from 300 to 350 g. SAH was induced in different ways: under light ether anaesthesia, 200 μl of autologous blood was injected either into the cisterna magna, or right lateral ventricle, or the basal subarachnoid space through the soft palate, or into the subarachnoid space over the right cerebral convexity. The latter, the cortical SAH model, ensured the most consistent common pattern as concerns the extent and location of the blood clot. In the other groups there were wide variations in this respect. For the ANP experiments the cortical SAH model was chosen, as it ensured the most standardizable SAH with least brain damage, and therefore appropriate for quantitative studies. Details of the procedures were published earlier. Briefly, a burr hole was drilled over the right cerebral convexity, 2 mm caudal from the coronal and 2 mm lateral from the sagittal sutures. Care was taken to keep the dura mater intact. The burr hole was sealed with bone wax, and the dura was pierced with a 25-gauge needle. A total of 200 μl of autologous blood was drawn from the tail; for control, 0.9% or cellfree autologous serum was injected into the left lateral ventricle, with the following stereotactic co-ordinates: A 5.3, L 1.8, V 3.1 mm below the cortical surface under light ether anaesthesia. The proper placement of the tip of the cannula had been tested in a previous experiment by injecting Evans blue dye into the ventricle. 10 μl of sodium chloride solution (0.9%) was chosen as the vehicle for the administration of ANP. Any influence of the icv. injection of 10 μl of 0.9% NaCl solution on the brain sodium and water contents was separately controlled. The doses of 2 and 5 μg of ANP were chosen on the basis of the results of previous experiments. One week prior to SAH induction, a cannula for chronic use was inserted under pentobarbital (60 mg/kg) anaesthesia into the left lateral ventricle, with the following stereotactic co-ordinates: A 5.3, L 1.8, V 3.1 mm below the cortical surface. The cannula was fixed with acrylic. No blood-brain barrier (BBB) damage to Evans blue was found around the cannula by the time of SAH induction. ANP was administered first at the time of SAH induction and was repeated 90 minutes later, i.e. 90 minutes before the animals were sacrificed.

**Estimation of Brain Water and Electrolytes**

Brain sodium, potassium, and water contents were determined either 90 minutes or 3 hours after induction of SAH by weighing the hemispheres of the SAH side before and after drying at 110°C for 20 hours, after which the ash was dissolved in 5 ml of 3 mol/L HNO₃ and diluted 10-fold with de-ionized water. Sodium was determined at 330.3 nm and potassium at 404.4 nm with a Perkin-Elmer 306 atomic absorption spectrophotometer in an air-acetylene flame. The slit width was 0.7 and 2 nm, respectively.

**Estimation of Serum Osmolality and Electrolytes**

Serum samples drawn at the end of the experiments were diluted with de-ionized water and subjected to ion determination under optimal analytical conditions. Osmolality was determined by the freezing point depression method. Statistical analyses were made using the two-tailed Student’s t-test.

**Experimental Protocols**

There were four control groups: 1a: “Unoperated”; 1b: “icv. administration of 10 μl of 0.9% NaCl”; 1c: “icv. administration of 2 μg ANP”; 1d: “icv. administration of 5 μg ANP”. There were 8 groups with SAH: Group 2: “90-minutes SAH” + sham-icv. procedure; Group 3: “3-hour SAH” + sham-icv. procedure; Group 4: “90-minutes SAH” + icv. administration of 10 μl of 0.9% NaCl; Group 5: “3-hour SAH” + icv. administration of 10 μl of 0.9% NaCl; Group 6: “90-minute SAH” + icv. administration of 2 μg of ANP; Group 7: “90-minute SAH” + icv. administration of 5 μg of ANP; Group 8: “3-hour SAH” + icv. administration of 5 μg of ANP followed by a repeated icv. injection of 5 μg of ANP into the chronic cannula 90 minutes before decapitation. All groups were investigated as described in Table 1.