Production of Cyclic Nucleotides from Normal Human Brain

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SUMMARY Constrictions of cyclic AMP and cyclic GMP in arterial and internal jugular venous (IJV) blood were determined at the resting wakeful state in thirty surgical patients without neurological deficits. The levels of cyclic AMP in artery and IJV were 32.1 ± 3.0 and 40.0 ± 4.1 pmol/ml (means ± standard errors), respectively, while those of cyclic GMP in artery and IJV were 12.2 ± 2.7 and 14.4 ± 3.0 pmol/ml, respectively. Concentrations of both cyclic nucleotides in IJV were significantly higher (P < 0.001) than those in artery. IJV-arterial differences for cAMP and cGMP were 7.9 ± 1.7 and 2.1 ± 0.5 pmol/ml, respectively. The results indicate that both cyclic nucleotides are constantly produced and released from the normal human brain.

Materials and Methods

Thirty patients (13 males and 17 females) aged 15 to 70, who underwent surgical operations were the subject of this study. Informed consent was obtained from the patients and their families before the study. They were premedicated with 30 mg of pentazocine, 0.5 mg of atropine and 50–100 mg of hydroxyzine administered intra-muscularly 30–45 minutes before the measurement. Simultaneous samplings of arterial and IJV blood were performed at the steady state of each subject before induction of anesthesia. The IJV blood was sampled from a catheter inserted in the jugular bulb.

At the same time, arterial blood was taken from the femoral artery. One ml of the sampled specimen was quickly transferred to a precooled 10 ml plastic tube containing 10 μl of 500 mM sodium edetate solution, with which it was mixed. Immediately after this procedure, the plasma was removed after centrifugation at 4°C. One hundred μl of the plasma was analysed for cAMP and cGMP by using the Yamasa cyclic AMP assay kit and cyclic GMP assay kit (commercially obtained from Yamasa Shoyu Co., Ltd., Choshi, Chiba, Japan) according to the ultrasensitive method of Honma et al.6

Student's t test on paired and unpaired data was used to compare mean values between groups and within groups respectively.

Results

Concentrations of cAMP in arterial and IJV blood were 32.1 ± 3.0 (mean ± standard error) and 40.0 ± 4.1 pmol/ml, respectively in the lightly premedicated wakeful state. The blood levels of cGMP in the arteries and IJV were 12.2 ± 2.7 and 14.4 ± 3.0 pmol/ml, respectively.

Figure 1 shows the values of cAMP and cGMP in the plasma of 30 patients before the induction of anesthesia. The levels of both cyclic nucleotides in the IJV were significantly higher (P < 0.001) than those in the arterial blood. IJV-arterial differences for cAMP and cGMP were 7.9 ± 1.7 and 2.1 ± 0.5 pmol/ml, respectively. In two subjects, however, the concentrations of the nucleotides were higher in the arteries than in the IJV (fig. 1).

Discussion

The contribution of organs or tissues to the regulation of cAMP and cGMP concentrations in the blood of dogs has been reported by Wehmann et al.11 They suggested that the kidneys and liver were the sites of removal of both cyclic nucleotides from the plasma and that the lungs and small intestine were the sites of net production of the nucleotides. However, study of cerebral venous concentrations of these cyclic nucleotides in animals and in normal man has not yet been carried out. Previously, cerebral arterio-venous differences for cAMP have been found in patients with cerebral infarction,7-9 and it has been suggested that cAMP is released or leaked from infarcted brain tissues. The present study, however, has shown that IJV-arterial differences of both cyclic nucleotides exist even in neurologically normal subjects, indicating their continuous release from the intact human brain. These findings indicate that the brain is one of the source organs for cAMP and cGMP in plasma. Thus, approximate net productions of cAMP and cGMP from normal human brain would amount to 395 and 110 pmol/100g brain/min, respectively, provided that mean cerebral
blood flow is 50 ml/100g/min in the present subjects. Welch et al\textsuperscript{8} has demonstrated that cerebral venous levels for cAMP are significantly higher (venous-arterial difference, 5.17 ± 1.54 pmol/ml) than arterial in 18 patients with recent cerebral infarction. They proposed two mechanisms for the venous-arterial difference for cAMP in their patients: release of cAMP into cerebral venous blood by damage to the blood brain barrier and an active transport mechanism as suggested by Cramer et al.\textsuperscript{2} IJV-arterial differences for cAMP (7.9 ± 1.7 pmol/ml) in our patients without neurological diseases are higher than those in the patients with cerebral infarction reported by Welch et al.\textsuperscript{8} It is conceivable that the lower levels for cerebral venous-arterial differences of cAMP in the stroke patients of Welch et al than those in our patients could be due to the disturbance in a transport mechanism of the cyclic nucleotide from brain into cerebral vein\textsuperscript{12} or to the different assay method adopted by them (a competitive binding protein).\textsuperscript{8} However, more detailed comparison study between normal and diseased brain must be carried out to solve these questions.

These intrinsic nucleotides might be formed in neurons or glia and travel through the blood-brain barrier. Another possible source for cAMP could be the cerebral vasculatures, since the concentrations of cAMP\textsuperscript{13} and adenylate cyclase\textsuperscript{14} in brain microvessels have been found to be regulated by the β-adrenergic agonists. From these facts it has been hypothesized that β-induced relaxation of vascular smooth muscle involves the cAMP\textsuperscript{15} or cGMP\textsuperscript{16} system in animals. Values of IJV-arterial differences of both cyclic nucleotides, however, varied considerably from subject to subject, and were negative in two subjects. The reason for this variability remains to be answered. Relationship between blood levels of these cyclic nucleotides and age or sex could not be found in the present study. Cramer et al.\textsuperscript{12} also found that cAMP levels in lumbar spinal fluid of patients with peripheral or central nervous system disease appeared unrelated to age and sex. Age-related differences in cAMP-dependent protein kinase activity have been also not observed in the rat or human cerebral cortex.\textsuperscript{17}

Thus, IJV-arterial differences of both cyclic nucleotides do not necessarily mean leakage from the infarcted brain area. It may rather indicate that both nucleotides are constantly released into cerebral blood stream from brain cells or cerebral vessels even under normal conditions.

References

Oxypurines in Cerebrospinal Fluid as Indices of Disturbed Brain Metabolism
A Clinical Study of Ischemic Brain Diseases

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SUMMARY Using a HPLC method the concentrations of oxypurines were simultaneously measured in CSF of patients with acute cerebrovascular lesions (CVL) and global cerebral ischemia (GCI) in an attempt to study disturbed brain metabolism during cerebral oxygen deprivation. In cerebral infarction both hypoxanthine and xanthine gradually increased from normal levels at admission to pathologically increased on the fourth day from onset of symptoms. There was no correlation between these substances and the clinical score but the maximum CSF-hypoxanthine concentration was significantly correlated to the maximum lesion volume determined by computerized tomography. In GCI the hypoxanthine-xanthine concentrations were considerably increased less than 20 hours from onset of unconsciousness but the initial levels did not predict the final outcome. These findings suggest that the end products of nucleotide degradation accumulate rapidly in acute cerebral hypoxia but more gradually in CVL probably due to growing local edema with subsequent local hypoxia. In controls and patients with CVL the CSF-urate concentrations were positively correlated to those of CSF-albumin. However, in CVL the increase of urate was relatively much more pronounced than the increase of albumin indicating that urate is a sensitive marker of dysfunction of blood-brain barrier.

NUMEROUS ANIMAL EXPERIMENTAL STUDIES have elucidated the changes in metabolism occurring during cerebral oxygen deprivation. As a consequence of decreased oxygen supply the high-energy phosphate utilization exceeds formation, causing a rapid fall of the intracellular adenine and guanine di- and trinucleotides in the brain. In parallel to these events an increase of corresponding monophosphonucleotides occurs in cerebral tissue. Further degradation may result in the end products of the purine metabolism, hypoxanthine and xanthine, while the absence or very low amounts of xanthine oxidase in the brain tissue of various mammalians is not consistent with the formation of urate. The appearance of urate in the cerebrospinal fluid (CSF) has therefore been attributed to a passive transport from the blood over the blood-brain barrier. In this study we have serially measured the oxypurines, i.e. hypoxanthine, xanthine and urate in CSF of patients with acute cerebrovascular lesions and global cerebral ischemia (GCI) in an attempt to elucidate disturbed brain metabolism in these conditions. For this purpose we have developed a HPLC (high pressure liquid chromatography) method which does not require extraction procedures of the CSF samples. The results obtained have been correlated to the magnitude of the lesions and the edema formation as estimated by repeated computerized tomography examinations. In patients with GCI we searched for a relationship between the CSF levels of the oxypurines during the initial period of cerebral deterioration and the final clinical outcome. The obtained data of hypoxanthine, xanthine and urate concentrations in CSF during normal and pathological conditions are discussed with respect to possible purine metabolic pathways in brain, elimination routes of xanthine and hypoxanthine and the possible role of urate as a sensitive marker of CSF-blood barrier function.

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