Alterations in Perivascular Dilatory Neuropeptides (CGRP, SP, VIP) in the External Jugular Vein and in the Cerebrospinal Fluid Following Subarachnoid Haemorrhage in Man


Departments of 1Neurosurgery, 2Biomedical Engineering, 3Anesthesia, University Hospital of Trondheim, Norway, 4Department of Neurology, Righospitalet, Copenhagen, Denmark, and 5Department of Internal Medicine, University Hospital of Lund, Sweden

Summary

A possible involvement of perivascular vasodilatory neuropeptides in subarachnoid haemorrhage (SAH) has been evaluated in man by measuring the levels of calcitonin gene related peptide (CGRP)-, substance P (SP)- and vasoactive intestinal peptide (VIP)-like immunoreactivity (LI) in the cranial venous outflow and in CSF in 34 patients admitted to the hospital after an acute SAH.

After operation with aneurysm clipping and nimodipine treatment, blood samples were taken from the external jugular vein (EJV) or cerebrospinal fluid (CSF) and analysed for neuropeptide levels with specific radioimmuno assays (RIA) during the postoperative course. The degree of vasoconstriction in the patients was monitored with Doppler ultrasound recordings bilaterally from the middle cerebral (MCA) and internal carotid arteries (ICA) following the EJV blood sampling every second day.

The mean value of all CGRP-LI measurements in EJV during the entire course of SAH (n = 20) revealed a significantly higher level as compared to controls. The highest CGRP-LI levels were found in patients with the highest velocity index values (vasospasm). The relationship Vmean MCA/Vmean ICA was used as an index of vasoconstriction. In patients with MCA aneurysms (n = 10), a significant correlation (r = 0.65, p < 0.05) was found between the vasospasm index and CGRP-LI levels. There were no changes observed in the SP- and VIP-LI levels. Alterations in cerebrovascular tone induced by changing arterial CO₂ tension or lowering of blood pressure (ketanserin infusion test) did not alter the levels of the perivascular peptides in the EJV.

In addition, CGRP-, SP-, VIP- and neuropeptide Y (NPY)-LI were analysed in CSF in the postoperative course after subarachnoid haemorrhage (SAH) in 14 patients. The CSF VIP-LI was lower in SAH than in control (p < 0.05). The CGRP-LI level was measurable in SAH CSF but not in CSF of controls. In individual patients with marked vasoconstriction increased levels of CGRP-LI (up to 14 pmol/L) and NPY-LI (up to 232 pmol/L) were observed.

The results of this study are in support of our hypothesis that there is an involvement of the sensory peptide CGRP in a dynamic reflex aimed at counterbalancing vasoconstriction in SAH.

Keywords: Calcitonin gene-related peptide; CGRP; subarachnoid haemorrhage; SAH; transcranial Doppler; trigemino-cerebrovascular system.

Introduction

The cerebral circulation is supplied with two types of vasodilatory perivascular nerves, parasympathetic and sensory fibres (for review see 55). The functional role of each of these systems is only partially known, particularly with regard to their role in pathophysiological conditions such as that which may follow subarachnoid haemorrhage (SAH) 24. In the parasympathetic nerves vasoactive intestinal peptide (VIP) co-exists with acetylcholine in fibres that originate in the sphenopalatine ganglion, otic ganglion and in small clusters of nerve cells at the base of the brain 16, 25, 35, 51, 57. The selective electrical stimulation of postganglionic cerebrovascular parasympathetic nerve fibres enhances cortical blood flow in the rat via an effect that is not blocked by cholinergic antagonists 50. This has been taken as evidence in favour of VIP being an important vasodilator in this system, besides nitric oxide 45. Administration of VIP results in dilatation of cerebral arteries, arterioles and veins, and increased cerebral blood flow and metabolism 27, 37, 42.

The perivascular sensory fibres store at least three peptides; tachykinins (substance P, neurokinin A) and calcitonin gene-related peptide (CGRP) (for review see 55). The fibres have their main origin in the trigeminal ganglion as revealed by immunocytochemistry and quantitative measurements of CGRP and substance P (SP), with a small contribution of fibres from the dorsal
root ganglion at the C2 level\textsuperscript{10, 41, 56}. This system has been called the trigemino-cerebrovascular system and can be activated experimentally by vasoconstriction induced by perivascular administration of noradrenaline, prostaglandin \textit{F}$_{2\alpha}$, \textit{BaCl}$_2$ or subarachnoid blood\textsuperscript{6, 7, 14, 40, 43, 46, 48}. These experiments have suggested an involvement of this system in a reflex aiming to restore cerebrovascular tone after induced vasoconstriction\textsuperscript{12}. It is well-known that SAH can be followed by vasospasm of cerebral arteries, albeit to a varying degree. It has been shown that there are significant modifications of content and secretion rate of classical neurotransmitters supplying the cerebrovascular system after SAH (for review, see \textsuperscript{24}). Thus, modification of the efficiency of the noradrenaline re-uptake mechanism has been shown to occur early during SAH-induced vasospasm\textsuperscript{43}. Furthermore, the sympathetic co-transmitter neuropeptide \textit{Y} (NPY) has been found to be reduced in the wall of cerebral vessels\textsuperscript{30}. Clinically there is data showing enhanced release of NPY\textsuperscript{33, 52}. Thus, the sympathetic nerves may participate in causing the constriction since both noradrenaline and NPY are strong vasoconstrictors. In relation to the counter-regulatory mechanisms immunocytochemical studies in animals have demonstrated gradual reduction of the perivascular content of substance \textit{p}\textsuperscript{26}, CGRP\textsuperscript{3, 6, 46} and VIP\textsuperscript{26} following SAH. This is supported by a marked loss of CGRP in the middle cerebral artery of humans after a fatal SAH\textsuperscript{7}.

The purpose of the present study was to provide further support for an involvement of perivascular vasodilator neuropeptides (CGRP, SP and VIP) following SAH in man and to examine whether such changes can be related to the clinical course and the haemodynamic changes seen with transcranial Doppler ultrasound.

**Methods**

The material consists of four groups of individuals; two groups of individuals following SAH, one with blood sampled from the external jugular vein (EJV) and one with samples taken from CSF, also two corresponding control groups were obtained (see below). In addition, a set of volunteers were studied to examine if alterations in cerebral blood flow are associated with changes in EJV neuropeptide profile.

**Patients**

Thirty-four patients with verified SAH were admitted to the Neurosurgical Department, University Hospital, Trondheim, Norway (\textit{n} = 20 for EJV study and \textit{n} = 14 for CSF analysis). On admission, the patients were classified according to Hunt and Hess\textsuperscript{29} (for EJV study 29–70 years of age, 14 females and 6 males, and for CSF analysis 33–74 years of age, 7 females and 7 males). The amount of cerebral blood was classified on CT scans\textsuperscript{15}. When three- or four-vessel cerebral angiography demonstrated an aneurysm, the patient was treated in accordance with departmental policy: early operation with aneurysm clipping, intensive care, slight hypervolaemia and intravenous infusion of the calcium entry blocker nimodipine (2 mg/h for ten days). Four of the patients were not operated upon. Neurological state and outcome were graded according to Glasgow Coma Scale\textsuperscript{34} and Glasgow Outcome Scale\textsuperscript{35}.

**Healthy Volunteers**

Healthy individuals without neurological disease (equal sex distribution, age range 30–65 years) were investigated with one blood sample from the EJV (\textit{n} = 14) or removal of lumbar CSF (\textit{n} = 12). The healthy volunteers gave informed consent and the procedure was approved by the Ethics Committees, University of Trondheim and University of Lund.

**Doppler Recordings**

Doppler recordings were performed at one to three day intervals in the first ten days, with velocity registrations from both middle cerebral arteries (MCA) and internal carotid arteries (ICA)\textsuperscript{11, 12}. The haemodynamic index (Vmean MCA/Vmean ipsilateral ICA) was used for quantitation\textsuperscript{22, 38, 39}. The equipment utilized during the study was a TC 2–64 B (EME, Germany) with 2 MHz probes for transcranial velocity recordings and 4 MHz for carotid velocity recordings. The doppler recordings were performed bilaterally in order to document the degree of vasoconstriction. These tests were all performed by one of us (RJ).

**Autoregulation and CO$_2$ Responses**

In ten other healthy volunteers (age 23–36 years; 4 men and 6 women), under local anaesthesia, a plastic cannula was introduced into the right radial artery and a catheter was placed in the right jugular vein with the tip in the jugular bulb. An intravenous cannula was placed in the brachial vein for infusion of ketanserin. The mean arterial blood pressure (MABP) was continuously measured invasively via the cannula in the radial artery. During the study ECG and pulse rate were monitored. The effect of inhalation of CO$_2$ (hypercapnia vasodilatation) and hyperventilation (hypocapnia vasoconstriction) were examined in order to analyse whether cerebrovascular flow changes \textit{per se} caused alterations in perivascular peptide levels in the external jugular vein (EJV). Blood samples were drawn before and at the end of a 3-min maximal hyperventilation period. After 10 min rest the CO$_2$ concentration was increased by the volunteer breathing air with 4% CO$_2$ for 3 min and 7% CO$_2$ for 3 min after which EJV blood samples were drawn. The detailed methodology, cerebral blood flow and characteristics of the subjects have been given in a separate study\textsuperscript{47}. The study protocol was approved by the local Ethical Committee, University of Copenhagen. In conjunction with increases in CO$_2$ cerebral blood flow increased significantly. In this study the effect of ketanserin, a 5-hydroxytryptamine receptor antagonist, was administered as a bolus dose of 10 mg followed by an infusion of 6 mg/h. After 1 hour infusion EJV blood samples were taken. Ketanserin did not cause any significant changes in cerebral blood flow but a slight reduction of the cerebral metabolic rate for oxygen\textsuperscript{67}. 