CSF and plasma levels of pro-opiomelanocortin-related peptides in reversible ischaemic attacks and strokes

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SUMMARY Plasma and CSF β-endorphin (β-EP), β-lipotropin (β-LPH) and ACTH levels were studied in a group of 25 patients who underwent reversible ischaemic attacks or completed strokes. CSF β-EP and β-LPH in ischaemic patients were higher than those of the control population, independently of both clinical reversibility of the cerebral damage, and the time lapse sampling and the acute event. The presence of a CT demonstrable lesion was related to the highest CSF β-EP levels. These data confirm an involvement of central opioid substances in the phenomena related to brain ischaemia. ACTH levels in the CSF did not differ from the controls; this finding further supports the concept of an independent central secretion of the different pro-opiomelanocortin-related peptides. The peripheral plasma concentrations of β-EP, β-LPH and ACTH, were, in contrast, within the normal range, confirming that CSF and plasma contents of pro-opiomelanocortin-related peptides are differently controlled and originate from different sources.

Endogenous opioid peptides, namely those of the pro-opiomelanocortin family, such as β-endorphin (β-EP), exert a large number of biological effects which are mainly mediated by CNS receptors. The role of these peptides in modulating nociceptive/adaptive responses is well known; in addition, it has recently been suggested that the opioid systems are involved in the exacerbation and reversal of focal neurological deficits of ischaemic origin in experimental models. Moreover, these properties of opioid ligands seem to occur independently of their role in the control mechanisms of cardiovascular functions.

As is known from the early studies during the 70s, focal ischaemia is accompanied by increased CSF content of neurotransmitters, mainly 5-hydroxytryptamine (5-HT) and norepinephrine. These changes have been attributed to an extensive and acute neuronal depletion since neither synthesis inhibition nor degradation blockade can avoid the neurotransmitter depletion due to ischaemia.

In vivo measurements of CSF β-EP and leucine-enkephalin in a patient with focal brain ischaemia revealed double the normal concentration of the former peptide, although leucine-enkephalin levels were unchanged. Analogous findings were obtained by the same authors in the ischaemic hemisphere of gerbils submitted to unilateral carotid ligation, whose neurological deficits were reversed by high doses of naloxone. Reports of studies are contradictory about the efficacy of naloxone administration in patients with ischaemic stroke, probably owing to different selection of patients, different times of administration and different doses used by various investigators.

As a further contribution to this topic the present study reports the results of plasma and CSF measurements of β-EP and the other pro-opiomelanocortin-related peptides, β-lipotropin (β-LPH) and ACTH, in a group of patients who underwent reversible ischaemic attacks, and completed strokes.

Subjects and methods

Twenty-five patients, 21 males and four females, aged
Table 1  Population characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>Territory involved</th>
<th>Previous transient ischaemic events</th>
<th>Prodromal or concomitant headache</th>
<th>Arterial hypertension</th>
<th>Signs and symptoms of systemic atherosclerosis</th>
<th>CT focal ischaemic</th>
<th>focal oedema</th>
<th>WMLA</th>
<th>Blood brain barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIs cases</td>
<td>right carotid</td>
<td>5</td>
<td>9/10</td>
<td>6/10</td>
<td>8/10</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 cases</td>
<td>left vertebral</td>
<td></td>
<td>1/10</td>
<td>3/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stokes cases</td>
<td>right carotid</td>
<td>6</td>
<td>10/10</td>
<td>5/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 cases</td>
<td>left vertebral</td>
<td></td>
<td>3/15</td>
<td>12/15</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

WMLA = white matter low attenuation.

33–79 yr (mean 57-76 ± 12) with recent onset of acute neurological deficits of ischaemic origin were studied. Twenty-two sex-matched subjects aged 35–76 yr (mean 49-7 ± 12-66) were taken as controls.

Beside clinical assessment, all the patients underwent standard noninvasive procedures, including EEG, Doppler sonography and serial CT scans. According to the severity and evolution of the clinical symptoms, patients were subdivided into those who had reversible ischaemic attacks (10 cases) and those with completed strokes (15 cases), without significant differences in age distribution between the two groups (55-2 ± 13-65 and 59-46 ± 10-93 yr respectively).

The main population characteristics are reported in Table 1. The CT findings refer to the examination performed within the week preceding or following the CSF sampling. Ischaemic oedema surrounding the focal lesions was present in 2/3 reversible ischaemic attack cases (1 carotid: 1 vertebral) and in 4/13 stroke cases (3 carotid: 1 vertebral). CT pictures of diffuse white matter low attenuation were observed in two stroke patients (one without CT focal lesions). One or more previous transient ischaemic episodes had occurred in 9/10 reversible ischaemic attack cases and in 10/15 stroke cases, with an interval of at least 3 months from the episode considered for the present investigation. Arterial hypertension and signs and symptoms of systemic atherosclerosis were present in 6 and 8/10 reversible ischaemic attacks and in 6 and 12/15 stroke patients. A prodromal or concomitant "migraine-like" headache associated with the ischaemic event we considered, was reported in six reversible ischaemic attacks and in three major events.

CSF and plasma samples were taken at time intervals ranging from 48 h to 60 days from the acute event. In particular, 5/10 reversible ischaemic attack and 6/15 stroke cases were studied within the 2nd week. All reversible ischaemic attack patients had a negative neurological examination at the time of sampling and had been on a standard hospital schedule for a nondisabled patient (meals at 7.00—12.00—18.00; sleep from 22.00 to 6.00). At the time of sampling 7/15 stroke cases were severely disabled score 8 according to Patten et al., while the remaining cases were less severely disabled (score 4-7); those cases tested after the 2nd week from symptoms onset were following a standard rehabilitation programme.

No patient had a previous history of psychiatric or endocrine disease, or had received centrally acting medications and/or dexamethasone during the week preceding the investigation. Among hypertensive patients only those sufficiently controlled with diuretics took part in the study. At the time of observation none had depression (score of less than 20 on the Hamilton Rating Scale), nor mental deterioration (Mini Mental State more than 22).

Control cases were selected among patients in whom neurological lesions had been suspected at entry, and who had undergone a set of clinical and instrumental investigations with negative final results. Cases were excluded if there was a history of major psychiatric disorder, alcohol or drug abuse, signs and symptoms of cerebral coronary and/or peripheral atherosclerosis, arterial hypertension.

Sampling procedures and assays

CSF samples were obtained in all the patients and controls by lumbar puncture at 9 am, after overnight fasting and 24 h bed rest. One ml aliquots were immediately freeze dried and stored under nitrogen. The integrity of the blood-brain barrier was evaluated in all the patients and controls, according to Schip and Felgenhauer, measuring the CSF-plasma albumin gradient.

Heparinised blood samples (500 kIU/ml aprotinin added) were taken before CSF collection, after 30 minutes of saline infusion. Plasma obtained by centrifugation was stored at -20°C. β-EP and β-LPH radioimmunoassays were performed after silicic acid plasma extraction (3 ml) and gel-chromatography of plasma extracts (or CSF reconstituted sample). According to elution profiles of cold β-EP and β-LPH, two 16 ml fractions discarding 6 ml in between were collected from a Sephadex column (1 · 5 x 45 cm), eluted with 0-1 M acetic acid, 0-01% bovine serum albumin. Fractions, containing β-LPH and β-EP respectively, were freeze-dried, redissolved in 0-4 ml phosphate buffer 0-05 M, pH 7-4, and submitted to specific radioimmunoassays. The percent recovery of CSF samples was 88-4 ± 11-1% and 89-7 ± 9-9% for β-LPH and β-EP respectively. Recovery of plasma samples was 70-1 ± 7-4% and 66-4 ± 8-4%. Synthetic β-EP was obtained from Organon (Oss, Holland), purified β-LPH and both anti-N-terminal and C-terminal β-LPH sera were generously supplied by Prof CH Li (San Francisco, CA, USA). Sensitivity of both radioimmunoassays was 1.1 fmol/tube. Details are reported elsewhere.

ACTH was measured by radioimmunoassays on phosphate buffer, pH 7-4, 0-04 M, redissolved CSF samples utilising both antisemum and reference hormone from NIADDK (Bethesda, MD, USA), and labelled molecule from CIS (Saluggia, Italy).
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Fig 1  β-EP, β-LPH and ACTH CSF levels (single values) plotted against the time lapse between sampling and the acute event.

Fig 2  β-EP, β-LPH and ACTH levels (M ± SE) in the CSF of RIA (10 cases), strokes (15 cases) and controls (22 cases).
Results

β-EP and β-LPH CSF levels of all ischaemic patients (89.3 ± 7.1; 45.1 ± 5.2 M ± SE) were significantly higher (p < 0.01) than those of the control population (65.4 ± 9.0; 27.3 ± 2.7). The difference was maintained even when the patients were subdivided into the two clinical subgroups, since, as shown in fig 1, β-EP and β-LPH CSF levels of reversible ischaemic attack (97.4 ± 11.1; 43.2 ± 4.5) and stroke (84.3 ± 9.4; 46.4 ± 8.2) patients remained significantly higher (p < 0.01; p < 0.05, respectively) than those of the controls. On the contrary, ACTH CSF values did not show differences either between patients (9.5 ± 1.7) and controls (8.0 ± 1.4), or between the clinical subgroups of ischaemic episodes (reversible ischaemic attack, 9.2 ± 2.1, stroke, 9.7 ± 2.6).

When the opioid and ACTH levels were plotted against the time intervals between sampling and symptoms onset (fig 2), no correlations were found (β-EP r = 0.003; β-LPH r = -0.01; ACTH r = -0.1). The same was true when considering the blood brain barrier damage, a variable that, in the population as a whole, seemed not to influence the distribution of the elevated opioid levels (maintained: β-EP 92.6 ± 7.5; β-LPH 49.9 ± 6.4; ACTH 6.0 ± 0.3; damaged: β-EP 86 ± 7.1; β-LPH 39.9 ± 3.4; ACTH 13.3 ± 2.2).

The β-EP CSF values of the patients were correlated (p < 0.05) with the presence (103.8 ± 7.5) or absence (78.3 ± 5.2) of CT focal ischaemic lesions. No correlation, however, was observable when β-LPH and ACTH levels were considered.

Considering the territory involved, previous transient ischaemic events, arterial hypertension and the signs and symptoms of systemic atherosclerosis, no statistical significances were found. In particular, no difference appeared when comparing the mean values of patients with prodromal or concomitant headache associated with the ischaemic event (β-EP 84 ± 17.0; β-LPH 53.1 ± 10.1; ACTH 7.5 ± 1.6) with those without (β-EP 56.1 ± 12.8; β-LPH 46.1 ± 6.1; ACTH 5.6 ± 0.4).

Plasma concentrations of the three peptides are reported in table 2; there was no difference between patients and controls, even when the two clinical subgroups of ischaemic episodes were considered.

Discussion

These data demonstrate that focal cerebral ischaemia is associated with increased β-EP and β-LPH CSF levels, which appear to be independent of both the clinical reversibility of the cerebral damage and the time lapse between sampling and the acute event. Moreover, the maintained integrity of the blood brain barrier does not interfere with the opioid levels either in reversible ischaemic attack and in stroke subgroups.

These findings agree and enlarge the preliminary observations by Hosobuchi's group, demonstrating an involvement of central opioid substances in the phenomena related to brain ischaemia, even if there are conflicting data in humans about naloxone reversible ischaemia. Moreover, it is of interest that ACTH CSF content remains unaffected; this could suggest that the raised β-EP and β-LPH concentrations are a selective consequence of the ischaemic injury and further support the concept of an independent central secretion of proopiomelanocortin-related peptides.

Several hypotheses could be advanced in order to explain the β-EP increase after focal brain ischaemia. The opioid release could be stimulated by general hypoxia, acidosis and hypercapnia, as can occur in the peripheral circulation. Otherwise, bearing in mind the positive action of 5-HT on pituitary and hypothalamic β-EP content, the increase of both opioids could be a phenomenon secondary to the augmentation of 5-HT release in the CSF after cerebral ischaemia. Lastly, the increased opioid levels in CSF could be related to diachisis, a neurogenic phenomenon that is thought to influence cerebral blood flow and metabolism in areas far from the focal lesion. Discrepancies between the severity of the clinical symptoms and the CT findings are commonly found in cerebrovascular ischaemic disorders, even when the patients with positive CT findings for focal ischaemia showed the highest β-EP levels.

As far as the peripheral plasma concentrations are
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cancerned, neither ACTH nor β-EP, nor β-LPH plasma levels of patients differ from the control group. These results are in agreement with observations by Fallis et al. and further confirm that CSF and plasma content of pro-opiomelanocortin-related peptides are differently controlled, originate from different sources and are not linked by any relationship.

In conclusion, focal cerebral ischaemia, either reversible or permanent, is accompanied by high CSF β-EP and β-LPH levels, independently of the clinical picture. The occurrence of a demonstrable CT focal lesion is the factor which directly correlates with the maximal rise of β-EP, regardless of possible alterations in the blood brain barrier.

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