

Migraine biomarkers in cerebrospinal fluid: a systematic review and meta-analysis

Dongen, R.M. van; Zielman, R.; Noga, M.J.; Dekkers, O.M.; Hankemeier, T.; Maagdenberg, A.M.J.M. van den; ... ; Ferrari, M.D.

Citation

Dongen, R. M. van, Zielman, R., Noga, M. J., Dekkers, O. M., Hankemeier, T., Maagdenberg, A. M. J. M. van den, ... Ferrari, M. D. (2016). Migraine biomarkers in cerebrospinal fluid: a systematic review and meta-analysis. *Cephalalgia*, *37*(1), 49-63. doi:10.1177/0333102415625614

Version:	Not Applicable (or Unknown)
License:	Leiden University Non-exclusive license
Downloaded from:	https://hdl.handle.net/1887/43666

Note: To cite this publication please use the final published version (if applicable).





Migraine biomarkers in cerebrospinal fluid: A systematic review and meta-analysis

Cephalalgia 0(0) 1–15 © International Headache Society 2016 Reprints and permissions: sagepub.co.uk/journalsPermissions.nav DOI: 10.1177/0333102415625614 cep.sagepub.com



Robin M van Dongen¹, Ronald Zielman¹, Marek Noga², Olaf M Dekkers^{3,4}, Thomas Hankemeier², Arn MJM van den Maagdenberg^{1,5}, Gisela M Terwindt¹ and Michel D Ferrari¹

Abstract

Objective: To perform a meta-analysis of migraine biomarkers in cerebrospinal fluid (CSF) and of corresponding blood concentrations.

Methods: We conducted a systematic search for studies that measured biochemical compounds in CSF of chronic or episodic migraineurs and non-headache controls. Subsequent searches retrieved studies with blood measurements of selected CSF biomarkers. If a compound was assessed in three or more studies, results were pooled in a meta-analysis with standardised mean differences (SMD) as effect measures.

Results: Sixty-two compounds were measured in 40 CSF studies. Most important results include: increased glutamate (five studies, SMD 2.22, 95% CI: 1.30, 3.13), calcitonin gene-related peptide (CGRP) (three studies, SMD: 3.80, 95% CI: 3.19, 4.41) and nerve growth factor (NGF) (three studies, SMD: 6.47, 95% CI: 5.55, 7.39) in chronic migraine patients and decreased β -endorphin (β -EP) in both chronic (four studies, SMD: -1.37, 95% CI: -1.80, -0.94) and interictal episodic migraine patients (three studies, SMD: -1.12, 95% CI: -1.65, -0.58). In blood, glutamate (interictal) and CGRP (chronic, interictal and ictal) were increased and β -EP (chronic, interictal and ictal) was decreased.

Conclusions: Glutamate, β -EP, CGRP and NGF concentrations are altered in CSF and, except for NGF, also in blood of migraineurs. Future research should focus on the pathophysiological roles of these compounds in migraine.

Keywords

Migraine, biomarkers, cerebrospinal fluid, meta-analysis

Date received: 2 October 2015; revised: 23 November 2015; accepted: 7 December 2015

Introduction

Migraine is a prevalent episodic brain disorder (1). The World Health Organisation (WHO) rates migraine as one of the most disabling chronic disorders (2). Despite extensive research over the last decades, migraine pathophysiology is not completely understood (3). Although several compounds (e.g. calcitonin generelated peptide (CGRP), glutamate and serotonin) have been implicated in migraine pathophysiology, our understanding of the biochemistry of migraine is still limited (4,5). Identification and validation of biochemical biomarkers might help us in uncovering pathophysiological processes involved in migraine, which in turn might lead to diagnostic tests or new therapeutic strategies (6,7).

The field of biochemical biomarker research is expanding rapidly. Promising biomarkers have been discovered for brain disorders such as Alzheimer's disease, narcolepsy, and Parkinson's disease (8–10). Cerebrospinal fluid (CSF) is believed to reflect biochemical changes in the brain and therefore is the body fluid of primary interest for brain disorders (11). Although many small studies have analysed biochemical

R.M.D. and R.Z. contributed equally to this manuscript.

Corresponding author:

Robin M. van Dongen, Leiden University Medical Centre, Department of Neurology, P.O. 9600, 2300 WB Leiden, the Netherlands. Email: r.m.van_dongen@lumc.nl

¹Department of Neurology, Leiden University Medical Centre, the Netherlands

²Division of Analytical Biosciences, Leiden Academic Centre for Drug Research, the Netherlands

³Department of Clinical Epidemiology, Leiden University Medical Centre, the Netherlands

⁴Department of Clinical Epidemiology, Aarhus University Hospital, Denmark

⁵Department of Human Genetics, Leiden University Medical Centre, the Netherlands

changes in CSF from migraine patients, results were often inconsistent and have not led to pathophysiological and diagnostic biomarkers. However, the literature has never been systematically reviewed with quantitative synthesis of the evidence. With this first meta-analysis we aimed to identify biochemical migraine biomarkers which show consistent changes in CSF and to assess whether these changes are also present in blood.

Methods

Search strategy, study selection and eligibility criteria

We conducted and reported the review process in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (12). We performed an electronic search for published studies up to 16 August 2014 in MEDLINE, EMBASE and Web of Science on biochemical findings in CSF of migraine patients. Medical Subject Heading (MeSH) terms and free text terms were collated with the assistance of research librarians at the Leiden University Medical Centre. The full search string can be found in Supplement 1.

Two investigators (R.M.D. and R.Z.) independently assessed titles and abstracts to determine potential eligibility. Disagreement was resolved by discussion. The same investigators independently assessed the full-text articles of potentially relevant studies to verify if eligibility criteria were met, and to evaluate whether the results were adequately reported.

We included case-control studies and case-crossover studies (same patients studied in between and during migraine attacks), in which one or more endogenous compounds (metabolites, peptides, proteins) were quantified in CSF samples from migraine patients. Case reports were not considered eligible. Publications on pharmacological trials were excluded if no endogenous compounds were measured at baseline. Studies not written in English, conference abstracts, editorials and letters were also not eligible. Reference lists of articles eligible for full-text review and relevant reviews were additionally searched for potentially relevant studies.

Subsequent search for studies on blood concentrations of selected biomarkers

To assess whether CSF biomarkers show similar results in blood, we performed in a second stage a literature search for published data on measurements in plasma and serum. We specifically searched for studies reporting blood concentrations of compounds which had shown consistent and significant differences in metaanalysis of CSF data. These additional blood studies were identified and selected by performing the same search and selection process as described for CSF studies. The full search string for blood is reported in Supplement 1. After study selection, data were extracted and subsequently included in study assessment and meta-analysis following the same methodology as for CSF.

Data extraction

Data extraction was performed by one investigator (R.M.D) using a standardised extraction form. A second investigator (R.Z.) was consulted if discussion was necessary. Information was extracted on: (1) study design; (2) study population characteristics (sample size, age, gender, medication, comorbidity and other potential confounders) and study groups definition (diagnostic criteria, presence of migraine attack during sampling, presence of chronic migraine component); (3) sampling methods (fasting, timing and storage temperature), measurement methods and data analysis; and (4) concentrations of endogenous compounds (metabolites, peptides, proteins) in study (sub)groups, including statistical parameters. To obtain relevant missing information of studies included for meta-analysis, we attempted to contact corresponding authors twice via email.

Risk of bias assessment

To assess risk of bias, we adapted the Newcastle-Ottowa Scale (13) (Supplement 1). We considered definition of cases and controls to be adequate when published criteria were used for diagnosis of migraine patients. Selection of cases was adequate when patients were representative for the defined migraine type (no severe comorbidity or clinical reasons to sample body fluids). Selection of controls was adequate when controls were sampled from the same population as the cases. Comparability between cases and controls was assessed based on gender- and age-matching of study groups (either by design or analysis). Studies adequately describing sampling and measurement methods and performing measurements according to validated analytical methods were considered to have low risk of measurement bias. One investigator (R.M.D.) assessed selection and comparability, two investigators (R.M.D. and M.N.) assessed the description and validity of the measurements, and a third investigator (R.Z.) was contacted if discussion was necessary.

Group definition

We divided case-control comparisons into episodic migraine *versus* controls and chronic migraine *versus* controls. If there was no evidence that migraine patients

had a chronic component, we classified them as episodic migraine. Findings in episodic migraine were further classified based on migraine state: interictal and ictal. Migraine with aura patients and migraine without aura patients were grouped, because results were often not reported separately for these groups. When applicable, ictal versus interictal findings, from case-control and from case-crossover studies, were compared.

Meta-analysis: Pooling of results and statistical procedures

We used standardised mean differences (SMDs) with their 95% confidence interval (CI) as the main effect measure. Compound concentrations were analysed in meta-analysis if data were available from three or more studies for one of the defined group comparisons. The way we have dealt with missing data, irregularities in the data, and pooling of data was in accordance with approaches described by the Cochrane Collaboration (14) (Supplement 1). For quantitative synthesis, we used the inverse variance method. We applied a random-effects model by default given the expected clinical heterogeneity between studies. However, as the between-study variation cannot be estimated reliable in case of <5 studies, we applied a fixed-effects model in these instances. Homogeneity of effect sizes was assessed using the I^2 statistic and by visual inspection of forest plots. To examine the effect of inclusion of clear heterogeneous studies, we applied a sensitivity analysis to assess their specific effect on the overall effect size. For statistical analysis we used RevMan 5.2 (Cochrane IMS, Baltimore, MD, USA).

Results

Study selection and study characteristics

The selection of CSF studies is depicted in the flowchart (Figure 1). A total of 1197 unique articles were identified, of which 40 were considered eligible (38 case-



Figure 1. Flowchart of CSF study selection process.

^aStudies could be excluded for more than one eligibility criterion. Therefore, overlaps exist between these categories. CSF: cerebrospinal fluid.

Study characteristics	Studies	Risk of bias assessment	Studies
Publication year		Selection	Adequate
≤ 1960	I (3%)	Definition of cases	29 (73%)
1961–1980	7 (18%)	Selection of cases	20 (50%)
1981–2000	15 (38%)	Definition of controls	15 (39 %) ^a
≥ 2001	17 (43%)	Selection of controls	6 (16%) ^a
Study design		Comparability	
Case-control	38 (95%)	Matching for age and gender	8 (21%) ^a
Case-crossover only	2 (5%)	Matching for other factors	4 (11%) ^a
Migraine types and states		Measurements	
Episodic migraine	22 (58%) ^a	Measurement description	22 (55%)
lctal state	19 (50%) ^a	Validation of measurement technique	21 (53%)
Interictal state	13 (34%) ^a		
Mixed state	3 (8%) ^a		
Chronic migraine	16 (42%) ^a		
Control types			
Healthy	7 (18%)ª		
Spinal anaesthesia	4 (11%) ^a		
Diagnostic lumbar puncture ^b	15 (39%) ^a		
Other neurological diseases	9 (24%) ^a		

Table 1. Summary of study characteristics and risk of bias assessment of CSF studies.

Risk of bias assessment: number of studies which were assessed as adequate for the corresponding item. ^aTotal of 38 studies (excluding two casecrossover studies since no controls were present). ^bControls underwent a diagnostic lumbar puncture and, retrospectively, CNS disorders were excluded by original researchers after which samples were used as control samples. CSF: cerebrospinal fluid.

control studies and two case-crossover studies). Investigator agreement on title and abstract screening, before consensus, was $\kappa = 0.72$. Episodic migraine patients were sampled for 22 case-control studies and chronic migraine patients for 16 case-control studies. The number of cases ranged from 4 to 60 (average: 24) and the number of controls from 5 to 108 (average: 24). Description of individual study characteristics can be found in the electronic supplementary table. Twelve CSF studies were finally included in meta-analyses on compounds that were measured in multiple studies. The subsequent search for blood studies on selected CSF biomarkers is illustrated in Supplementary Figure S1.

Risk of bias assessment

Most CSF studies (73%) applied adequate diagnostic criteria (Table 1); 11 studies that did not report the use of diagnostic criteria were published before introduction of the International Classification of Headache Disorders (ICHD-I) (15). Criteria for chronic migraine (Silberstein (16) and ICHD second edition (ICHD-II) revision (17)) were applied by all but four studies on chronic migraine (75%).

Migraine cases were not always deemed representative for the diagnosed migraine type because lumbar punctures were performed to exclude other neurologic diseases (five studies), migraine patients were admitted to the hospital for unstated reasons (four studies) or because recruitment of cases was not clearly reported (11 studies) (Table 1). Controls often had lumbar punctures for other purposes than migraine patients; either for other diagnostic purposes (13 studies) or before spinal anaesthesia (four studies). Based on available cohort descriptions, only six studies recruited cases and controls from the same population, of which four studies were sampled from the general population. For 15 studies it was explicitly stated that controls had no personal history of migraine (Table 1). Furthermore, a minority of studies (eight studies) adjusted for age and gender.

Sampling and measurement methods were adequately described in 22 studies (55%; Table 1). The older publications especially lacked full and clear descriptions of methods. Measurement techniques were considered to be (partially) validated in 21 studies. Quantitation characteristics (precision, accuracy and limit of detection) were often not reported.

Biochemical findings

In total, 62 unique compounds have been measured in CSF from migraine patients (Table 2) (18–55). Frequently measured compounds (in three or more

Table 2. Overview of published biochemical measurements in CSF from migraine patients.

NEUROTRANSMITTER SYSTEMS Glutamatergic system 7 1^{10} 1^{21} 1^{22} 1^{22} 1^{23}		Studies N =	Chronic migraine	Episodic migraine Interictal	Episodic migraine Ictal
Glutamate 7 1 ¹⁸ 1 ¹⁹ 1 ²⁰ 1 ²¹ 1 ²² ud 2 ²² ud 2 ²³ 1 ⁴⁸ ud 2 ³ Glutamate 1 1 ²³ (†) ²³ (†) ²³ (†) ²³ Glutamate 1 1 ²³ (†) ²³ (†) ²³ (†) ²³ Glutamate 1 1 ²³ (†) ²³ (†) ²³ (†) ²³ Glutamate 1 1 ²³ (†) ²³ (†) ²³ (†) ²³ Strotonergic system 2 $=^{23}$ $=^{38} =^{29}$ (†) ²³ $=^{31} a^{29} a^{2$	NEUROTRANSMITTER SYSTEMS				
Glutamine $7 + 7^{10} + 7^{10} + 7^{10} + 7^{10} + 7^{10} + 10^{1$	Glutamatergic system	_	. 18 . 19 . 20 . 21 . 22 23	. 22	. 24 . 22
Glutamine 1 f^{22} (f) ²² (f) ²² (f) ²² Spring (f) ²² (f) ²² (f) ²² Sarctonergic system S-hydroxyndoleacetic acid 4 $=^{23}$ (f) ²² (f) ²² (f) ²⁵ (f) ²	Glutamate	7	\uparrow^{10} \uparrow^{17} \uparrow^{20} \uparrow^{21} \uparrow^{22} u.d. ²³	u.d. ²³	\uparrow^{24} u.d. ²³
Gycine (1) e^{2x} (1) e^{2x} (1) e^{2x} (1) e^{2x} (1) e^{2x} Service regressions (1) e^{2x} (1	Glutamine	 	↑ ²³	$(\uparrow)^{23a}$	$(\uparrow)^{23a}$
Servicency is system Tryptophan 2 $=^{22}$ $(-)^{23} (+)^{27}$ $(-)^{25} =^{24} (-)^{23} (+)^{27}$ Tryptophan 2 $=^{28} =^{39}$ $\uparrow^{28} +^{29}$ Tryptophan 2 $=^{28} =^{39}$ $\uparrow^{28} +^{29}$ Homovanillic acid 4 $=^{30} =^{33}$ $=^{25} (=)^{23} (=)^{27} +^{25} (=)^{23} (=)^{27}$ \uparrow^{31} \uparrow^{31} Tryrosine 1 $=^{31}$ Epinephrine 1 $=^{31}$ \uparrow^{34} -dhydroxyphenylacetic acid 1 $=^{33}$ u.d. ²³ $=^{32} (=)^{23} (=)^{27} (=)^{2$	Glycine	I	123	$(\uparrow)^{23a}$	$(\uparrow)^{23a}$
S-hydroxyindolacetic acid 4 = $^{-23}$ (-) ⁻³ = $^{-3}$ (-) ⁻³ (-)	Serotonergic system		22		
Tryptophan 2 $=^{45} =^{47}$ \uparrow^{46} \uparrow^{47} (\uparrow^{47} \uparrow^{47} bydroxyprypramine 1 $u.d.^{23}$ Dopaminergic system Homovanillic acid 4 $=^{30} =^{23} =^{25} (=)^{23} (=)^{27} =^{35} (=)^{3} (=)^{37} (=)^{37}$ \uparrow^{31} \uparrow^{31} Epinephrine 1 $=^{31}$ Epinephrine 1 $=^{32}$ GABAergic system γ -Aminobutyric acid 4 $=^{33}$ $u.d.^{23}$ $u.d.^{34}$ $u.d.^{35}$ $u.d.^{23}$ ($\uparrow\uparrow^{34} (\uparrow\uparrow)^{35}$ $u.d.^{23}$ Cholinergic system γ -Aminobutyric acid 4 $=^{33}$ $u.d.^{23}$ $u.d.^{34}$ $u.d.^{35}$ $u.d.^{23}$ ($\uparrow\uparrow^{34} (\uparrow\uparrow)^{35}$ $u.d.^{23}$ Cholinergic system γ -Aminobutyric acid 4 $=^{33} u.d.^{23}$ $u.d.^{24}$ $u.d.^{35}$ $u.d.^{23}$ ($\uparrow\uparrow^{34} (\uparrow\uparrow)^{36}$ NEUROPEPTIDES Endogenous opioids β -endorphin 5 $\downarrow^{30} \downarrow^{37} \downarrow^{38} \downarrow^{39}$ $\downarrow^{37} \downarrow^{38} \downarrow^{40}$ \downarrow^{40} β -indorphin 2 $\downarrow^{37} \downarrow^{38} =^{37} =^{38}$ α -Nacetyl-[b-endorphin 1 \uparrow^{40} Met-enkephalin 1 \uparrow^{410} Met-enkephalin 1 \uparrow^{416} γ -Nacetyl-[f-endorphin 2 $\downarrow^{27} \uparrow^{18} \uparrow^{47}$ Neuropeptides Substance P 2 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptides Calcitonin gene-related peptide 3 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptides ENDOCANNABINODS Anandamide 1 \uparrow^{43} Palmictylethanolamide 1 \downarrow^{43} Palmictylethanolamide 1 \uparrow^{43} Palmictylethanolamide 1 \uparrow^{43} Palmictylethanolamide 1 \uparrow^{47} Paration neerosis factor-sipha 2 \uparrow^{47} Turon neerosis factor-sipha 2 \uparrow^{47} Palmictylethanolamide 1 \downarrow^{47} Palmictylethanolamide 1 \downarrow^{47} Palmictylethanolamide 1 \downarrow^{47} Palmictylethanolamide 1 \downarrow^{49} Palmictylethan	5-hydroxyindoleacetic acid	4	=23	$(=)^{23} = \frac{26}{29} (=)^{23} (\uparrow)^{27a}$	$(=)^{23} = {}^{26} (=)^{23} (\uparrow)^{27a}$
5-hydroxytrypamine I u.d. ²³ Dopaminergic system Homovanille acid 4 $= {}^{30} = {}^{33} = {}^{25} (=)^{23} (=)^{27} = {}^{25} (=)^{23} (=)^{27}$ 1^{21} 1^{2	Tryptophan	2	22	$=^{20} =^{27}$	$\uparrow^{20}\uparrow^{27}$
Dopaninergic system 4 = $^{30} = ^{23}$ = $^{25} (=)^{21} (=)^{27}$ = $^{25} (=)^{21} (=)^{27}$ Homovanilic acid 4 $= ^{30} = ^{23}$ $= ^{25} (=)^{21} (=)^{27}$ $= ^{31} (=)^{31} (=)^{37}$ Adhlydroxyphenylacetic acid 1 $= ^{31} (=)^{23} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{27} (=)^{28} (=)^{27} (=)^{28} (=)$	5-hydroxytryptamine	I	u.d. ²³		
Homovanilic acid 4 =30 =30 =50 =50 =20 =20 =20 =30 <td>Dopaminergic system</td> <td></td> <td>20 22</td> <td>25 22 27</td> <td>25 22 27</td>	Dopaminergic system		20 22	25 22 27	25 22 27
3.4-diffydroxyphenylacetic acid i $1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 $	Homovanillic acid	4	= ³⁰ $=$ ²³	$=^{25} (=)^{23} (=)^{27}$	$=^{25} (=)^{23} (=)^{27}$
Tyrosine I $=^{41}$ Epinephrine I $=^{32}$ SABAergic system $=^{32}$ $=^{32}$ GABAergic system I $=^{32}$ Cholinergic system I Image: System of the system of the system of the system of the system Image: System of the system of the system Acceylcholine I Image: System of the system Image: System of the	3,4-dihydroxyphenylacetic acid	I			³¹
Epinephrine I $= 1 = 1 = 1 = 1$ Norepinephrine $= 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 3^{32}$ $\frac{GABAergic system}{2} = 3^{32}$ $\frac{GABAergic system}{2} = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1 = 1 = 1 = 1$ $\frac{GABAergic system}{2} = 1 = 1 = 1 = 1 = 1 = 1 = 1 = 1 = 1 = $	Tyrosine	I			=31
Norepirephrine I $=^{22}$ GABAergic system $=^{27}$ Acesple holinergic system $(\uparrow)^{34} (\uparrow)^{35}$ u.d. ²³ Cholinergic system $(\uparrow)^{36}$ Acesple holine I $(\uparrow)^{36}$ u.d. ²³ Acesple holine I $(\uparrow)^{36}$ NEUROPEPTIDES Endogenous opioids β -endorphin $5 \downarrow^{30} \downarrow^{37} \downarrow^{38} \downarrow^{39}$ $\downarrow^{37} \downarrow^{38} \downarrow^{40}$ \downarrow^{40} β -lipotropin $2 \downarrow^{37} \downarrow^{38} =^{27} =^{38}$ $a^{-N-accepl-\beta-endorphin}$ $1 \uparrow^{30}$ Enkephalins' $1 \uparrow^{30}$ $\gamma^{-N-accepl-\beta-endorphin}$ $1 \uparrow^{30}$ $\gamma^{-N-accepl-\beta-endorphin}$ $1 \uparrow^{41c}$ Tachykinin neuropeptides Substance P $2 \uparrow^{18} \uparrow^{42}$ Neurokinin A $1 \uparrow^{18}$ Other neuropeptides Calcitonin gene-related peptide $3 \uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptide Y $2 = 4^{40} = 4^{40} \uparrow^{44}$ Somatostain $2 \downarrow^{45} = 4^{40} \downarrow^{40}$ γ^{40} Orexin-A $1 \uparrow^{46}$ ENDOCANNABINOIDS Anandamide $1 \downarrow^{43}$ Palmitoplethanolamide $1 \downarrow^{43}$ Palmitoplethanolamide $1 \downarrow^{43}$ Palmitoplethanolamide $1 \downarrow^{43}$ Palmitoplethanolamide $1 \downarrow^{42} \uparrow^{42}$ Nerve growth factor $3 \uparrow^{20} \uparrow^{21} \uparrow^{42}$ Gila cell-derived neurotrophic factor $1 \downarrow^{45}$ CYTOKINES Timmer nercosis factor-alpha $2 \downarrow^{47}$ Timmer netrosis factor-alpha $2 \uparrow^{47}$ Timmer netrosis factor-alpha $2 \uparrow^{47}$ Timmer netrosis factor-alpha $2 \uparrow^{47}$ Calcitor alpha \uparrow^{46} Calcitor alpha \uparrow^{46} Calcitor hereotrophic factor $2 \uparrow^{40} \uparrow^{21}$ Gila cell-derived neurotrophic factor $1 \downarrow^{45}$ CYTOKINES	Epinephrine	I			$=^{32}$
GABAergic system γ -Aninobutyric acid 4 $=^{33}$ u.d. ²³ u.d. ³⁴ u.d. ³⁵ u.d. ²³ $(\uparrow)^{34}$ $(\uparrow)^{35}$ u.d. ²³ Cholinergic system	Norepinephrine	I			$=^{32}$
γ-Aminobutyric acid 4 = 33 u.d. 23 u.d. 34 u.d. 35 u.d. 23 (↑) 34 (↑) 35 u.d. 23 Cholinergic system Acetylcholine 1 (↑) 36 NEUROPEPTIDES Endogenous opioids β -endorphin 5 $\sqrt[3]{30}$ $\sqrt[37]{38}$ $\sqrt[39]{39}$ $\sqrt[37]{38}$ $\sqrt[40]{40}$ β -lipotropin 2 $\sqrt[37]{38}$ $= \frac{37}{=38}$ a -N-acetyl- β -endorphin 1 $\sqrt[7]{38}$ a -N-acetyl- β -endorphin 1 $\sqrt[7]{38}$	GABAergic system				
Choinergic systemI(\uparrow) ³⁶ AcceytholineI(\uparrow) ³⁶ NEUROPEPTIDES I(\uparrow) ³⁶ Bendorphin5 $\downarrow^{30} \downarrow^{37} \downarrow^{38} \downarrow^{39}$ $\downarrow^{37} \downarrow^{38} \downarrow^{40}$ \downarrow^{40} β -lendorphin2 $\downarrow^{37} \downarrow^{38} \downarrow^{39}$ $\downarrow^{37} \downarrow^{38} \downarrow^{40}$ \downarrow^{40} β -lendorphin2 $\downarrow^{37} \downarrow^{38}$ $=^{37} =^{38}$ $=^{37} =^{38}$ $=^{37} =^{38}$ Adrenocorticotropic hormone2 $\downarrow^{37} =^{38}$ $=^{37} =^{38}$ $=^{37} =^{38}$ $=^{37} =^{38}$ α -N-acceyt)- β -endorphin1 \uparrow^{30} $=^{28b}$ \downarrow^{28} Metenkephalins'1 $=^{28b}$ \downarrow^{28} Metenkephalin1 $\uparrow^{41}e$ $\downarrow^{41}e$ Tachykinin neuropeptides2 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ $=^{40} \uparrow^{41} e^{41} e^{41}$ Neurokinin A1 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ $=^{40} =^{40} \uparrow^{44}$ Other neuropeptides2 $\downarrow^{45} =^{43} =^{40} e^{40} e^{40$	γ-Aminobutyric acid	4	$=^{33}$ u.d. ²³	u.d. ³⁴ u.d. ³⁵ u.d. ²³	$(\uparrow)^{34} (\uparrow)^{35}$ u.d. ²³
AcetylcholineI(\uparrow) ³⁶ NEUROPEPTIDES Endogenous opioids β -endorphin5 j^{30} j^{37} j^{38} 4^{40} β -lipotropin2 j^{37} j^{38} $=^{37}$ $=^{38}$ $=^{37}$ Adrenocorticotropic hormone2 $=^{37}$ $=^{38}$ $=^{38}$ $=^{37}$ $=^{38}$ <	Cholinergic system				
NEUROPEPTIDESEndogenous opioids β -endorphin5 $\sqrt{30} \sqrt{37} \sqrt{38} \sqrt{37} \sqrt{38} \sqrt{40}$ $\sqrt{40}$ β -lipotropin2 $\sqrt{37} \sqrt{38}$ $=^{37} - 38$ Δ drenocorticotropic hormone2 $2^{37} - 38$ $=^{37} - 38$ α -N-acetyl- β -endorphin1 γ^{30} $=^{37} - 38$ α -N-acetyl- β -endorphin1 γ^{40} $=^{37} - 38$ α -N-acetyl- β -endorphin1 γ^{18} $=^{28b}$ β -indykinin neuropeptides1 γ^{18} $=^{40}$ β -dictionin gene-related peptide3 γ^{45} $=$	Acetylcholine	I			$(\uparrow)^{36}$
Endogenous opioidsβ-endorphin5 $\sqrt{30}$ $\sqrt{37}$ $\sqrt{38}$ $\sqrt{40}$ $\sqrt{40}$ β-lipotropin2 $\sqrt{37}$ $\sqrt{38}$ $=^{37}$ $=^{38}$ $=^{37}$ $=^{38}$ Adrenocorticotropic hormone2 $=^{37}$ $=^{38}$ $=^{37}$ $=^{38}$ $=^{37}$ $=^{38}$ α -N-acetyl-β-endorphin1 \uparrow^{30} $=^{28b}$ $\sqrt{28}$ $=^{28b}$ $\sqrt{28}$ Met-enkephalins'1 \uparrow^{18} \uparrow^{41c} $=^{28b}$ $\sqrt{28}$ Met-enkephalin1 \uparrow^{18} \uparrow^{41c} $=^{28b}$ $\sqrt{28}$ Neuropeptides1 \uparrow^{18} \uparrow^{41c} $=^{40}$ \uparrow^{41} Other neuropeptide3 \uparrow^{18} \uparrow^{42} \uparrow^{43} $=^{40}$ Neuropeptide Y2 $=^{40}$ $=^{40}$ \uparrow^{40} Orexin-A1 \uparrow^{45} $=^{40b}$ \downarrow^{40} Orexin-A1 \uparrow^{43} $=^{40}$ \downarrow^{40} Palmitoylethanolamide1 \uparrow^{43} $=^{40}$ \downarrow^{40} Palmitoylethanolamide1 \uparrow^{43} $=^{40}$ \downarrow^{40} Palmitoylethanolamide1 \downarrow^{43} $=^{40}$ \downarrow^{40} Nerve growth factor3 \uparrow^{20} \uparrow^{21} \downarrow^{42} Brain-derived neurotrophic factor1 \downarrow^{45} $=^{40}$ \downarrow^{48} CYTOKINES1 \downarrow^{46} $=^{48}$ \downarrow^{48}	NEUROPEPTIDES				
β -endorphin5 \downarrow^{30} \downarrow^{37} \downarrow^{37} \downarrow^{37} \downarrow^{30} \downarrow^{40} β -lipotropin2 \downarrow^{37} \downarrow^{38} $=^{37}$ $=^{38}$ $=^{37}$ $=^{38}$ Adrenocorticotropic hormone2 $=^{37}$ $=^{38}$ $=^{37}$ $=^{38}$ $=^{37}$ $=^{38}$ Adrenocorticotropic hormone2 $=^{37}$ $=^{38}$ $=^{37}$ $=^{38}$ $=^{37}$ $=^{38}$ Adrenocorticotropic hormone1 \uparrow^{30} $=^{28b}$ \downarrow^{28} $=^{40}$ $=^{40}$ $=^{40}$ Met-enkephalin1 \uparrow^{18} \uparrow^{41c} $=^{40}$ $=^{40}$ \uparrow^{41} Substance P2 \uparrow^{18} \uparrow^{42} \uparrow^{43} $=^{40}$ $=^{40}$ \uparrow^{41} Neuropeptides3 \uparrow^{18} \uparrow^{42} \uparrow^{40} \downarrow^{40} \downarrow^{40} Orexin-A1 \uparrow^{45} $=^{40}$ \downarrow^{40} \downarrow^{40} Orexin-A1 \uparrow^{43} $=^{40}$ \downarrow^{40} Palmitoylethanolamide1 \downarrow^{43} $=^{40}$ $=^{40}$ Rein-derived neurotrophic factor2 \uparrow^{20} \uparrow^{21} <td< td=""><td>Endogenous opioids</td><td></td><td></td><td></td><td></td></td<>	Endogenous opioids				
$\begin{array}{c c c c c c c } \beta - lipotropin & 2 & \downarrow^{37} \downarrow^{38} & =^{37} - ^{38} \\ \hline Adrenocorticotropic hormone & 2 & -^{37} - ^{38} & -^{37} - ^{38} \\ \hline Adrenocorticotropic hormone & 2 & -^{37} - ^{38} & -^{37} - ^{38} \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & \uparrow^{30} & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & \uparrow^{30} & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & \uparrow^{30} & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & \uparrow^{30} & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & 1 & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta - endorphin & & & & & \\ \hline & -^{N-acetyl-} \beta $	β -endorphin	5	$\downarrow^{30}\downarrow^{37}\downarrow^{38}\downarrow^{39}$	$\downarrow^{37}\downarrow^{38}\downarrow^{40}$	↓ ⁴⁰
Adrenocorticotropic hormone 2 $=^{37} =^{38}$ $=^{37} =^{38}$ α -N-acetyl- β -endorphin 1 \uparrow^{30} 'Enkephalins' 1 $=^{28b}$ \downarrow^{28} Met-enkephalin 1 \uparrow^{41c} Tachykinin neuropeptides Subtance P 2 $\uparrow^{18} \uparrow^{42}$ Neurokinin A 1 \uparrow^{18} Other neuropeptides Calcitonin gene-related peptide 3 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptide Y 2 $=^{40} =^{40} \uparrow^{44}$ Somatostatin 2 $\downarrow^{45} =^{40b} \downarrow^{40}$ Orexin-A 1 \uparrow^{46} ENDOCANNABINOIDS Anandamide 1 \downarrow^{43} Palmitoylethanolamide 1 \uparrow^{43} 2-Arachidonoylglycerol 1 $u.d.^{43}$ Neurogrowth factor 3 $\uparrow^{20} \uparrow^{21} \uparrow^{42}$ Brain-derived neurotrophic factor 1 \downarrow^{45} CYTOKINES Tumor necrosis factor-alpha 2 \uparrow^{47} $u.d.^{48}$	β-lipotropin	2	$\downarrow^{37}\downarrow^{38}$	$=^{37} =^{38}$	
α -N-acetyl- β -endorphinI \uparrow^{30} 'Enkephalins'I $=^{28b}$ \downarrow^{28} Met-enkephalinI \uparrow^{41c} Tachykinin neuropeptidesSubstance P2 \uparrow^{18} Substance P2 \uparrow^{18} \downarrow^{41c} Neurokinin AI \uparrow^{18} \downarrow^{41c} Other neuropeptides3 \uparrow^{18} \downarrow^{41} Calcitonin gene-related peptide3 \uparrow^{18} \downarrow^{40} Orexin-AI \uparrow^{45} $=^{40}$ \downarrow^{40} Orexin-AI \uparrow^{46} \downarrow^{43} PalmitoylethanolamideI \downarrow^{43} \downarrow^{43} PalmitoylethanolamideI \downarrow^{43} \downarrow^{41} PalmitoylethanolamideI \downarrow^{43} \downarrow^{41} PalmitoylethanolamideI \downarrow^{43} \downarrow^{41} PalmitoylethanolamideI \downarrow^{42} \downarrow^{42} Brain-derived neurotrophic factor2 \uparrow^{20} \uparrow^{21} Glai cell-derived neurotrophic factor1 \downarrow^{45} \downarrow^{46} CYTOKINESI \downarrow^{45} ITumor necrosis factor-alpha2 \uparrow^{47} IInterleykin-I receptor antaponist1I \downarrow^{46}	Adrenocorticotropic hormone	2	$=^{37}=^{38}$	$=^{37}=^{38}$	
"Enkephalins"I $=2^{28b}$ \downarrow^{28} Met-enkephalinI \uparrow^{41c} \uparrow^{41c} Tachykinin neuropeptidesSubstance P2 \uparrow^{18} \uparrow^{42} Neurokinin AI \uparrow^{18} \uparrow^{42} \uparrow^{43} Other neuropeptides3 \uparrow^{18} \uparrow^{42} \uparrow^{43} Outre reuropeptide Y2 $=^{40}$ $=^{40}$ Somatostatin2 \downarrow^{45} $=^{40b}$ \downarrow^{40} Orexin-AI \uparrow^{46} Image: Second Sec	α -N-acetyl- β -endorphin	I.	1 ³⁰		
Met-enkephalinI \uparrow^{41c} Tachykinin neuropeptidesI \uparrow^{18} ISubstance P2 \uparrow^{18} INeurokinin AI \uparrow^{18} IOther neuropeptidesI \uparrow^{18} ICalcitonin gene-related peptide3 \uparrow^{18} INeuropeptide Y2=40=40Somatostatin2 \downarrow^{45} =40bOrexin-AI \uparrow^{46} IENDOCANNABINOIDSI \downarrow^{43} IAnandamideI \downarrow^{43} IPalmitoylethanolamideI \downarrow^{43} IPalmitoylethanolamideI \downarrow^{43} IPalmitoylethanolamideI \downarrow^{43} IOrexy growth factor3 \uparrow^{20} \uparrow^{21} Sumator Fore FilterIIINerve growth factorI \downarrow^{20} IGlial cell-derived neurotrophic factorI \downarrow^{20} CYTOKINESIIITumor necrosis factor-alpha2 \uparrow^{47} U.d. ⁴⁸ Interleukin-I receptor antagonistIIInterleukin-I r	'Enkephalins'	I		= ^{28b}	↓ ²⁸
Tachykinin neuropeptidesSubstance P2 $\uparrow^{18} \uparrow^{42}$ Neurokinin A1 \uparrow^{18} Other neuropeptides3 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Calcitonin gene-related peptide3 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptide Y2 $=^{40} = =^{40} \uparrow^{44}$ Somatostatin2 $\downarrow^{45} = =^{40b} = \downarrow^{40}$ Orexin-A1 \uparrow^{46} ENDOCANNABINOIDSI \downarrow^{43} Anandamide1 \downarrow^{43} Palmitoylethanolamide1 \downarrow^{43} 2Arachidonoylglycerol1 $u.d.^{43}$ Nerve growth factor3 $\uparrow^{20} \uparrow^{21} \uparrow^{42}$ Brain-derived neurotrophic factor1 \downarrow^{45} CYTOKINESI \downarrow^{47} Tumor necrosis factor-alpha2 \uparrow^{47} Und 4 ⁸ I \uparrow^{49}	Met-enkephalin	I		∱ ⁴¹ c	
Substance P2 $\uparrow^{18} \uparrow^{42}$ Neurokinin AI $\uparrow^{18} \uparrow^{42}$ Neurokinin AI $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Calcitonin gene-related peptide3 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptide Y2 $=^{40} = =^{40} \uparrow^{44}$ Somatostatin2 $\downarrow^{45} = =^{40b} = \downarrow^{40}$ Orexin-AI \uparrow^{46} ENDOCANNABINOIDSIAnandamideIPalmitoylethanolamideI2Arachidonoylg/cerolIu.d.^{43}NEUROTROPHINSNerve growth factor3Paini-derived neurotrophic factor1J \downarrow^{45} CYTOKINESTumor necrosis factor-alpha2Interleukin-L receptor antagonistIIIInterleukin-L receptor antagonistIIIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LIInterleukin-LInterleukin-LInterleukin-LInterleukin-LInterleukin-LInterleukin-L <tr< td=""><td>Tachykinin neuropeptides</td><td></td><td></td><td></td><td></td></tr<>	Tachykinin neuropeptides				
Neurokinin AI \uparrow^{18} Other neuropeptidesCalcitonin gene-related peptide3 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptide Y2 $=^{40} =^{40} \uparrow^{44}$ Somatostatin2 $\downarrow^{45} =^{40b} \downarrow^{40}$ Orexin-AI \uparrow^{46} ENDOCANNABINOIDSI \downarrow^{43} AnandamideI \uparrow^{43} PalmitoylethanolamideI \uparrow^{43} 2ArachidonoylglycerolIu.d. ⁴³ NEUROTROPHINSI $\downarrow^{20} \uparrow^{21} \uparrow^{42}$ Brain-derived neurotrophic factor2 $\uparrow^{20} \uparrow^{21} \uparrow^{42}$ Glial cell-derived neurotrophic factorI \downarrow^{45} CYTOKINESI \downarrow^{47} Tumor necrosis factor-alpha2 \uparrow^{47} Und receptor antagonistI \downarrow^{48}	Substance P	2	↑ ¹⁸ ↑ ⁴²		
Other neuropeptidesCalcitonin gene-related peptide3 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptide Y2 $=^{40}$ $=^{40} \uparrow^{44}$ Somatostatin2 \downarrow^{45} $=^{40b}$ Orexin-A1 \uparrow^{46} ENDOCANNABINOIDSAnandamide1 \downarrow^{43} Palmitoylethanolamide1 \uparrow^{43} 2-Arachidonoylglycerol1 $u.d.^{43}$ Neuro growth factor3 $\uparrow^{20} \uparrow^{21} \uparrow^{42}$ Brain-derived neurotrophic factor1 \downarrow^{45} CYTOKINESI \downarrow^{47} Tumor necrosis factor-alpha2 \uparrow^{47} u.d. ⁴⁸	Neurokinin A	I	↑ ¹⁸		
Calcitonin gene-related peptide3 $\uparrow^{18} \uparrow^{42} \uparrow^{43}$ Neuropeptide Y2 $=^{40}$ $=^{40} \uparrow^{44}$ Somatostatin2 \downarrow^{45} $=^{40b}$ \downarrow^{40} Orexin-A1 \uparrow^{46} Image: Second Secon	Other neuropeptides				
Neuropeptide Y2 $=^{40}$	Calcitonin gene-related peptide	3	$\uparrow^{18}\uparrow^{42}\uparrow^{43}$		
Somatostatin2 \downarrow^{45} $=^{40b}$ \downarrow^{40} Orexin-AI \uparrow^{46} ENDOCANNABINOIDSAnandamideI \downarrow^{43} PalmitoylethanolamideI \uparrow^{43} PalmitoylethanolamideI \uparrow^{43} 2-ArachidonoylglycerolIu.d.^{43} NEUROTROPHINS VNerve growth factor3 \uparrow^{20} Brain-derived neurotrophic factor2 \uparrow^{20} Glial cell-derived neurotrophic factorI \downarrow^{45} CYTOKINESTumor necrosis factor-alpha21 \downarrow^{47} u.d.^{48}Interleukin-I receptor antagonistI \downarrow^{48}	Neuropeptide Y	2		=40	= ⁴⁰ ↑ ⁴⁴
Orexin-AI 1^{46} ENDOCANNABINOIDSAnandamideI 1^{43} PalmitoylethanolamideI 1^{43} PalmitoylethanolamideI 1^{43} 2-ArachidonoylglycerolI $u.d.^{43}$ NEUROTROPHINSI 1^{20} 1^{21} 1^{42} Nerve growth factor3 1^{20} 1^{21} Brain-derived neurotrophic factor1 1^{45} CYTOKINESI 1^{45} Tumor necrosis factor-alpha2 1^{47} Interleukin-L receptor antagonistI 1^{47}	Somatostatin	2	↓ ⁴⁵	= ^{40b}	↓ ⁴⁰
ENDOCANNABINOIDSAnandamideI \downarrow^{43} PalmitoylethanolamideI \uparrow^{43} PalmitoylethanolamideI \downarrow^{43} 2-ArachidonoylglycerolIu.d. ⁴³ NEUROTROPHINSNerve growth factor3 \uparrow^{20} Paini-derived neurotrophic factor2 \uparrow^{20} Glial cell-derived neurotrophic factorI \downarrow^{45} CYTOKINESTumor necrosis factor-alpha21 \downarrow^{47} u.d. ⁴⁸ Interleukin-I receptor antagonistI \downarrow^{48}	Orexin-A	I	∱ ⁴⁶		·
AnandamideI \downarrow^{43} PalmitoylethanolamideI \uparrow^{43} 2-ArachidonoylglycerolIu.d.^{43}NEUROTROPHINSNerve growth factor3 $\uparrow^{20} \uparrow^{21} \uparrow^{42}$ Brain-derived neurotrophic factor2 $\uparrow^{20} \uparrow^{21}$ Glial cell-derived neurotrophic factorI \downarrow^{45} CYTOKINESTumor necrosis factor-alpha21 \downarrow^{47} u.d.^{48}Interleukin-I receptor antagonistI \downarrow^{48}	ENDOCANNABINOIDS		1		
PalmitoylethanolamideI \uparrow^{43} 2-ArachidonoylglycerolIu.d.^{43} NEUROTROPHINS I $\downarrow^{20} \uparrow^{21} \uparrow^{42}$ Nerve growth factor3 $\uparrow^{20} \uparrow^{21} \uparrow^{42}$ Brain-derived neurotrophic factor2 $\uparrow^{20} \uparrow^{21}$ Glial cell-derived neurotrophic factorI \downarrow^{45} CYTOKINESTumor necrosis factor-alpha21 \downarrow^{47} u.d.^{48}Interleukin-I receptor antagonistI \downarrow^{48}	Anandamide	I	↓ ⁴³		
2-Arachidonoylglycerol I u.d. ⁴³ NEUROTROPHINS Nerve growth factor 3 $\uparrow^{20} \uparrow^{21} \uparrow^{42}$ Brain-derived neurotrophic factor 2 $\uparrow^{20} \uparrow^{21}$ Glial cell-derived neurotrophic factor I \downarrow^{45} CYTOKINES Tumor necrosis factor-alpha 2 \uparrow^{47} u.d. ⁴⁸ Interleukin-1 receptor antagonist I \downarrow^{48}	Palmitoylethanolamide	I	↑ ⁴³		
NEUROTROPHINSNerve growth factor3 \uparrow^{20} \uparrow^{21} \uparrow^{42} Brain-derived neurotrophic factor2 \uparrow^{20} \uparrow^{21} Glial cell-derived neurotrophic factorI \downarrow^{45} CYTOKINESTumor necrosis factor-alpha2 \uparrow^{47} u.d. ⁴⁸ Interleukin-I receptor antagonistI \downarrow^{48} \uparrow^{48}	, 2-Arachidonovlglycerol	1	u.d. ⁴³		
Nerve growth factor3 \uparrow^{20} \uparrow^{21} \uparrow^{42} Brain-derived neurotrophic factor2 \uparrow^{20} \uparrow^{21} Glial cell-derived neurotrophic factorI \downarrow^{45} CYTOKINESTumor necrosis factor-alpha2 \uparrow^{47} Interleukin-I receptor antagonistI \uparrow^{48}	NEUROTROPHINS				
Brain-derived neurotrophic factor 2 $\uparrow^{20} \uparrow^{21}$ Glial cell-derived neurotrophic factor 1 \downarrow^{45} CYTOKINES Tumor necrosis factor-alpha 2 \uparrow^{47} u.d. ⁴⁸ Interleukin-1 receptor antagonist 1 \uparrow^{48}	Nerve growth factor	3	↑ ²⁰ ↑ ²¹ ↑ ⁴²		
Glial cell-derived neurotrophic factor I 45 CYTOKINES Tumor necrosis factor-alpha 2 1 ⁴⁷ u.d. ⁴⁸ Interleukin-L receptor antagonist I 1 ⁴⁷	Brain-derived neurotrophic factor	2	²⁰ ¹ ²¹		
CYTOKINES Tumor necrosis factor-alpha 2 1 1^{47} u.d. ⁴⁸ 1	Glial cell-derived neurotrophic factor	-	↓ 45 ↓ 45		
Tumor necrosis factor-alpha 2 1 ⁴⁷ u.d. ⁴⁸	CYTOKINES	-	•		
Interleukin-L receptor antagonist	Tumor necrosis factor-alpha	2	↑ ⁴⁷		u.d. ⁴⁸
	Interleukin-1 receptor antagonist	-			∱ ⁴⁸

(continued)

Table 2. Continued.

	Studies N =	Chronic migraine	Episodic migraine Interictal	Episodic migraine lctal
Monocyte chemotactic protein-l	I			↑ ⁴⁸
Transforming growth factor Beta I	I			↑ ⁴⁸
Interleukin-10	I			u.d. ⁴⁸
Interleukin-Iβ	I			u.d. ⁴⁸
Interleukin-4	I			u.d. ⁴⁸
METAL IONS				
Calcium (ionised)	I		=49	=49
Calcium (total)	I		=49	=49
Magnesium (total)	I		=49	=49
Potassium	I		=49	=49
Sodium	I		= ^{49b}	↑ ⁴⁹
OTHER				
Nitrite products (NO, NO ₂ –, NO ₃ –)	3	$\uparrow^{18}\uparrow^{43}$		= ^{44d}
Taurine	2	\uparrow^{23}	$(\uparrow)^{23a}$	$\uparrow^{50} (\uparrow)^{23a}$
Albumin	I		=27	=27
Aspartic acid	I			u.d. ²⁴
Chromogranin A	I			=44
Corticotropin-releasing hormone	I	↑ ⁴⁶		
Cortisol	I		↓ ⁵¹	
Cyclic adenosine monophosphate	I			1 ³⁵
Follicle-stimulating hormone	I		(↑) ⁵¹	
Guanosine 2',3'-cyclic phosphate	I	↑ ¹⁸		
Homocysteine (free)	I		=52	
Homocysteine (total)	I		↑ ⁵²	
Immunoglobulin G	I		=27	=27
Luteinizing hormone	I		(↑) ⁵¹	
Methionine	I		\downarrow^{52}	
Neuron-specific enolase	I			$(=)^{53}$
Phosphatidylcholine-specific phospholipase	CΙ		$=^{54b} =^{55}$	1,54
Prolactin	I		(↑) ⁵¹	

Published biochemical findings in migraine patients compared with controls. \uparrow = significantly elevated concentrations, \downarrow = significantly decreased concentrations, "=" = significant concentrations, between () = no statistical analysis reported, u.d. = undetectable concentrations reported for studied migraine group. ^aSignificant when interictal and ictal groups were pooled. ^bSignificant difference between ictal and interictal migraine patients reported. ^cMigraine state not reported. ^dNitric oxide (NO) was measured; not reported if NO₂⁻ and NO₃⁻ were also quantified. Excluding glucose from the routine CSF measurements (nine studies reported normal glucose concentrations in migraine patients). CSF: cerebrospinal fluid.

studies) are glutamate, β -endorphin (β -EP), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), CGRP and nerve growth factor (NGF). Forty-four compounds were measured only once. Meta-analysis showed that glutamate (Figure 2), β -EP (Figure 3), CGRP (Figure 4) and NGF (Figure 5) concentrations were consistently altered in CSF from migraine patients compared to controls; results will be discussed below, together with results on blood concentrations. For HVA there was insufficient quantitative data available for meta-analysis (CSF concentrations not reported separately for ictal and interictal patients) and for 5-HIAA CSF studies showed inconsistent effects (Supplementary Figure S2).

Glutamate (Figure 2)

Glutamate concentrations were increased in CSF from chronic migraine patients (SMD: 2.22, 95% CI: 1.30, 3.13) (18–22). In blood from interictal episodic migraine patients the pooled difference was not statistically significant (SMD: 1.08, 95% CI: -0.07, 2.22) (56–62). After exclusion of paediatric migraine patients (58), glutamate concentrations were increased in the

Chronic migraine																
	Cas	es					Con	trols						SMD (95% CI)	SMD (95% CI))
CSF	N=	Mean	SD	Age	Fem	МО	N=	Mean	SD	Age	Fem	н	Weight	Random-effects	T	
Sarchielli et al. 2002	25	2.7	0.5	46.5	18	NR	20	1.4	0.3	44.9	13	Ν	19.5%	3.02 [2.14, 3.89]		
Gallai et al. 2003	25	2.21	0.26	44.7	16	NR	20	1.00	0.25	44.6	13	Ν	18.7%	3.61 [2.64, 4.59]	-	_
Peres et al. 2004	30	0.25	0.186	42.3	14	NR	20	0.041	0.186	5 NR	NR	Ν	21.1%	1.10 [0.43, 1.77]		
Viera et al. 2007	19	0.289	0.177	42.9	13	NR	19	0.109	0.066	5 NR	NR	Ν	20.8%	1.32 [0.61, 2.03]		
Sarchielli et al. 2007 (I)	20	2.18	0.40	38.4	16	NR	20	1.37	0.30	41.6	15	Ν	20.0%	2.25 [1.44, 3.05]		
l ² = 85%																
Pooled estimate: Z = 4.75	109						99						100%	2.22 [1.30, 3.13]	•	
<i>P</i> < 0.0001																
Blood	No	studies p	oublishe	d												
Episodic migraine - Interio	ctal															
CSF	No	studies p	oublishe	d												
	Cas	es					Cont	rols						SMD (95% CI)		
Blood	N=	Mean	SD	Age	Fem	МО	N=	Mean	SD	Age	Fem	н	Weight	Random-effects		
Ferrari, MD et al. 1990	P 31	62.9	19.5	42.5	26	21	9	31.7	19.5	22.8	9	Υ	14.0%	1.57 [0.74, 2.39]		
Cananzi et al. 1995	P 57	15.80	8.38	28.5	28	25	19	14.60	7.76	35	9	Υ	14.6%	0.14 [-0.38, 0.66]	+	
D'Eufemia et al. 1997 (PM)	P 34	24.60	6.73	10.4	18	19	16	41.90	8.69	10.6	8	Υ	14.1%	-2.30 [-3.06, -1.54]		
Alam et al. 1998	P 89	481.9	126.1	NR	75	80	62	277.0	87.0	NR	43	Υ	14.8%	1.82 [1.44, 2.21]	-	
Vaccaro et al. 2007	P 50	35.4	8.1	35.5	33	25	20	20.7	4.3	38	12	Υ	14.4%	2.01 [1.39, 2.63]	-	
Ferrari, A et al 2009*	P 22	61.79	18.75	33.6	NR	22	24	9.36	2.10	33.3	20	Υ	13.5%	3.95 [2.93, 4.97]	_	_
Campos et al. 2013	S 45	153.7	68.6	37.3	44	33	16	121.5	59.2	31.2	15	Y	14.5%	0.48 [-0.10, 1.06]	-	
l ² = 96%																
Pooled estimate: Z = 1.93	328						166						100.0%	1.08 [-0.07, 2.22]	•	
<i>P</i> = 0.07																
Sensitivity analysis	Exclud	ina serui	m (S): l ²	² = 97%	6. Z=1.	70. P =	= 0.09							1.18 [-0.18, 2.54]		
	Exclud	ing pedia	atric mię	graine ((PM): I ²	= 92%	%, Z=3	.58, <i>P</i> =	0.0003					1.61 [0.73, 2.49]		
Episodic migraine - Ictal																
	Cas	es					Cont	rols						SMD (95% CI)		
CSF	N=	Mear	SD	Aae	Fem	МО	N=	Mean	SD	Aae	Fem	н	Weiaht	Unpooled		
Martinez et al. 1993	25	0.328	0.074	38/39	17/19	15	19	0.18	0.07	49/50	6/8	Ν	NA	2.01 [1.27, 2.75]	+	
	Cas	es					Cont	rols						SMD (95% CI)		
Blood	N=	Mear	n SD	Age	Fem	МО	N=	Mean	SD	Age	Fem	н	Weight	Unpooled		
Ferrari, MD et al. 1990	P 31	84.5	19.5	42.5	26	21	9	31.7	19.5	22.8	9	Υ	NA	2.65 [1.69, 3.62]		
Martinez et al. 1993	P 26	0.56	0.22	38/39	18/19	15	21	0.98	0.64	50.0	8	Ν	NA	-0.90 [-1.51, -0.30]	+	
															-2 0 2	4
															Lower Higher	
															-	



The squares represent effect sizes of the individual studies (size reflects the weight of the study) and the horizontal lines indicate the 95% confidence intervals (Cl). The filled diamonds represent the overall effect size (horizontal width indicates the 95% Cl). Age: mean age; Fem: number of females; MO: number of cases with migraine without aura; H: healthy controls; Y: yes; N: no; NR: not reported; P: plasma concentrations; S: serum concentrations; CSF: cerebrospinal fluid. *Migraine state is not explicitly reported for this study, the interictal state was assumed. PM: paediatric migraine patients and paediatric controls. Additional information on the handling of missing data (e.g. calculations, assumptions) can be found in the supplement.

remaining adult migraineurs (SMD: 1.61, 95% CI: 0.73, 2.49). Glutamate concentrations were increased in CSF from ictal patients (SMD: 2.01, 95% CI: 1.27, 2.75) (24). In blood, two studies showed clearly opposing results on ictal measurements and therefore we did not perform a meta-analysis (24,56). There are no studies on glutamate concentrations in blood from chronic

migraine patients and in CSF from interictal migraineurs.

β -Endorphin (Figure 3)

 $\beta\text{-}EP$ concentrations were decreased in CSF (SMD: – 1.37, 95% CI: –1.80, –0.94) (30,37–39) and blood

Chronic migraine					
	Cases		Controls	SMD (95% CI)	SMD (95% CI)
CSF	N= Mean S	SD Age Fem MO	N= Mean SD Age Fem H	Weight Fixed-effects	1
Genazzani et al. 1984	6 14.8 9	9.8 44.8 2 NR	15 86.1 37.0 38.1 NR Y	13.1% –2.13 [–3.31, –0.94] —	
Nappi et al. 1985 (I)	8 17.0 1	10.47 NR NR NR	30 86.1 50.92 NR NR Y	25.3% -1.47 [-2.33, -0.62]	
Nappi et al. 1985 (II)	14 17.79 1	10.34 NR NR NR	16 65.8 26.6 NR NR N	20.7% –2.26 [–3.20, –1.31] —	
Facchinetti et al. 1992	15 9.8 9	9.4 46 11 15	22 15.7 9.7 43 9 N	40.9% -0.60 [-1.27, 0.07]	
l ² = 70%					
Pooled estimate: Z = 6.23	43		83	100.0% -1.37 [-1.80, -0.94]	•
<i>P</i> < 0.0001					
	Cases		Controls	SMD (95% CI)	
Blood	N= Mean S	SD Age Fem MO	N= Mean SD Age Fem H	Weight Fixed-effects	
Nappi et al. 1985 (I)	P 11 4.7 2	2.3 NR NR NR	51 6.7 6.4 NR NR Y	38.5% -0.33 [-0.99, 0.32]	
Martignoni et al. 1989	P 25 4.79 2	2.60 43.1 16 25	10 7.35 2.66 NR NR Y	27.7% -0.96 [-1.73, -0.19]	
Misra et al. 2013	P 17 3.74 2	2.20 NR NR NR	20 6.68 2.93 37 NR Y	33.8% -1.10 [-1.80, -0.40]	—
l ² = 28%					
Pooled estimate: Z = 3.69	53		81	100.0% -0.76 [-1.17, -0.36]	•
<i>P</i> = 0.0002					
Episodic migraine - Inter	ictal		Controls	SMD (05% CI)	
CSE	Vases			Swid (93% CI)	
	N= Mean 3	SD Age Fem MO	N= Mean SD Age Fem F	Weight Fixed-effects	
Genazzani et al. 1984	5 31.5 3	3.5 39.4 1 5	15 86.1 37.0 38.1 NH Y	21.5% -1.60 [-2.75, -0.45]	
Nappi et al. 1985 (I)	7 25.7 1	10.05 NR NR 7	30 86.1 50.92 NR NR Y	37.2% –1.27 [–2.15, –0.39]	
Vecsei et al. 1992	13 79.3 1	12.44 37.6 13 13	11 88.8 12.9 41.7 4 N	41.2% –0.73 [–1.56, 0.11]	
12 001					
I ⁻ = 0%					
Pooled estimate: Z = 4.09	25		56	100.0% -1.12 [-1.65, -0.58]	•
P < 0.0001	Casaa		Controlo	SMD (05% CI)	
Direct	Cases	00 4 5 140			
Blood	N= Mean 3	SD Age Fem MO	N= Mean SD Age Fem F	i weight Fixed-effects	
Facchinetti et al. 1981	P 11 24.4 5	5.8 38.1 4 11	8 26.0 6.1 37.5 4 Y	6.6% -0.26 [-1.17, 0.66]	
Baldi et al. 1982	P 11 11.2 4	4.6 NR NR 11	12 12.6 4.5 NR 8 Y	8.2% -0.30 [-1.12, 0.53]	
Facchinetti et al. 1983 (PM) *	P 7 9.1 2	2.9 NR NR 7	6 5.6 8.8 NR NR Y	4.5% 0.52 [-0.60, 1.63]	
Fettes et al. 1985	P 33 12.3 1	17.3 34 27 22	29 20.9 17.2 NR 11 N	3 21.6% -0.49 [-1.00, 0.01]	
Nappi et al. 1985 (I) *	P 11 6.9 5	5.3 NR NR 11	51 6.7 6.4 NR NR Y	13.1% 0.03 [-0.62, 0.68]	
Facchinetti et al. 1986 (MM) *	P 8 7.5 3	3.0 NR 8 NR	3 9.2 1.7 NR NR N	3.0% -0.56 [-1.92, 0.80]	
Awaki et al. 1989	S 11 4.3 3	3.0 36.7 11 8	9 6.0 5.3 NR 9 Y	7.0% –0.39 [–1.28, 0.50]	
Facchinetti et al. 1989 (MM) *	P 9 10.0 2	2.5 30 9 9	6 9.61 1.83 28.5 NR Y	5.2% 0.16 [-0.87, 1.20]	
vd Helm et al. 1990 (PM) *	P 20 3.96 1	1.67 NR NR 19	20 5.75 3.43 NR NR Y	13.7% -0.65 [-1.29, -0.01]	
Vécsei et al. 1992	P 13 29.33 8	8.8 37.6 13 13	11 35.56 10.31 41.7 4 N	8.1% -0.63 [-1.46, 0.19]	
Battistella et al. 1996 (PM)	P 13 16.2 4	4.2 NR NR 13	17 21.3 4.6 11.2 6 Y	9.0% -1.12 [-1.90, -0.34]	
$l^2 = 0\%$					
Pooled estimate: Z = 3.31	147		172	100.0% -0.40 [-0.64, -0.16]	•
<i>P</i> = 0.0002					
Consitivity on chain	Evel of		R 0.00	0.007.007.01	
Sensitivity analysis	Excluding serum	n (S): I ² = 9%, Z=2.97,	P = 0.03	-0.39 [-0.65, -0.13]	
	Excluding pedia	atric migraine (PNI): I- =	: 0%, Z=2.25, P = 0.02	-0.32 [-0.59, -0.04]	
	Excluding mens	strual migraine (MM): I ²	= 8%, Z=3.20, P = 0.001	-0.42 [-0.68, -0.16]	•
Episodic migraine - Ictal					
	Cases		Controls	SMD (95% CI)	
CSF	N= Mean S	SD Age Fem MO	N= Mean SD Age Fem H	Weight Unpooled	
Vécsei et al. 1992	9 71.6 1	10.35 39.3 9 9	11 88.8 12.9 41.7 4 N	NA -1.39 [-2.40, -0.39]	
	Cases		Controls	SMD (95% CI)	
Blood	N= Mean S	SD Age Fem MO	N= Mean SD Age Fem H	Weight Unpooled	
	P 15 5.3	1.9 NR NR 15	12 12.6 4.5 NR 8 Y	47.2% -2.14 [-3.12, -1.16] -	
Baldi et al. 1982	P 9 28.00 6	6.66 39.3 9 9	11 35.56 10.31 41.7 4 N	52.8% -0.82 [-1.74, 0.11]	
Baldi et al. 1982 Vécsei et al. 1992					
Baldi et al. 1982 Vécsei et al. 1992					
Baldi et al. 1982 Vécsei et al. 1992 I ² = 73%					
Baldi et al. 1982 Vécsei et al. 1992 $I^2 = 73\%$ Pooled estimate: Z = 4.20	24		23	100% –1.44 [–2.11, –0.77]	•
Baldi et al. 1982 Vécsei et al. 1992 $I^2 = 73\%$ Pooled estimate: Z = 4.20 P < 0.0001	24		23	100% -1.44 [-2.11, -0.77]	<u> </u>

Figure 3. Forest plot of β -EP concentrations in migraine patients and controls.

The squares represent effect sizes of the individual studies (size reflects the weight of the study) and the horizontal lines indicate the 95% confidence intervals (CI). The filled diamonds represent the overall effect size (horizontal width indicates the 95% CI). β -EP: β -endorphin; Age: mean age; Fem: number of females; MO: cases with migraine without aura; H: healthy controls; Y: yes; N: no; NR: not reported; P: plasma concentrations; S: serum concentrations. *Migraine state is not explicitly reported for this study, the interictal state was assumed. PM: paediatric migraine patients and paediatric controls; MM: menstrual migraine. Additional information on the handling of missing data (e.g. calculations, assumptions) can be found in the supplement.

Chronic migraine			
	Cases	Controls	SMD (95% CI) SMD (95% CI)
CSF	N= Mean SD Age Fem MO	N= Mean SD Age Fem H Weight	Fixed-effects
Sarchielli et al. 2001	20 55.23 7.37 43.6 15 NR	20 11.35 2.58 42.1 13 N 10.3%	7.79 [5.89, 9.69]
Gallai et al. 2003	25 1.26 0.14 44.7 16 NR	20 0.78 0.10 44.6 13 N 36.5%	3.81 [2.79, 4.82]
Sarchielli et al. 2007 (II)	30 44.06 4.85 38.8 24 NR	20 29.37 4.67 36.3 13 N 53.2%	3.03 [2.19, 3.86]
l ² = 90%			
Pooled estimate: Z = 12.20	75	60 100.0%	3.80 [3.19, 4.41]
<i>P</i> < 0.0001			10701
	Cases	Controls	SMD (95% CI)
Blood	N= Mean SD Age Fem MO	N= Mean SD Age Fem H Weight	Fixed-effects
Gupta et al. 2009	P 7 0.94 0.17 NR NR NR	50 1.11 0.53 25.2 12/13 Y 9.8% -	-0.33 [-1.13, 0.46]
Jang et al. 2011	P 33 253.6 195.2 43.7 21 NR	36 136.2 92.5 44.3 19 Y 25.7%	0.77 [0.28, 1.26]
Cernuda et al. 2013	S 103 74.90 28.29 43.1 103 57	31 33.74 16.10 38.6 31 Y 31.2%	1.57 [1.13, 2.02]
Oterino et al. 2013	S 51 47.18 36.89 32.4 39 NR	35 41.78 41.94 31.4 23 Y 33.3%	0.14 [-0.29, 0.57]
l ² = 89%			
Pooled estimate: Z = 5.54	194	152 100.0%	0.70 [0.45, 0.95]
<i>P</i> < 0.0001			
Episodic migraine - Interict	al		
CSF	No studies published		
	Cases	Controls	SMD (95% CI)
Blood	N= Mean SD Age Fem MO	N= Mean SD Age Fem H Weight	Random-effects
Gallai et al. 1995 (PM)	P 75 37.46 50.9 15.9 32 45	30 38.2 35.6 15.1 15 NR 12.5% -	-0.02 [-0.44, 0.41]
Ashina et al. 2000	P 20 75.0 35.8 40 16 15	20 49.0 13.4 41 12 Y 8.8%	0.94 [0.29, 1.60]
Juhasz et al. 2003	P 15 18.4 6.58 41.9 15 NR	8 15.1 5.66 38.5 8 Y 6.4%	0.51 [-0.37, 1.38]
Fusayasu et al. 2007	P 95 19.0 9.1 30.1 77 54	52 13.4 4.4 29.2 39 Y 13.8%	0.72 [0.37, 1.06]
Fan et al. 2009 (PM)	P 66 113.6 219.9 NR 27 NR	22 52.4 26.3 NR 9 N 11.4%	0.32 [-0.17, 0.80]
Gupta et al. 2009	P 43 1.14 0.53 NR NR NR	50 1.11 0.53 25.2 12/13 Y 12.7%	0.06 [-0.35, 0.46]
Rodriguez et al. 2012	S 47 164.2 139.1 37.8 46 33	23 37.1 38.5 31.1 22 Y 10.7%	1.08 [0.55, 1.61]
Oterino et al. 2013	S 48 45.08 38.29 33.1 33 NR	35 41.78 41.94 31.4 23 Y 12.3%	0.08 [-0.35, 0.52]
Cernuda et al. 2013	S 43 46.37 15.21 44.4 43 NR	31 33.74 16.1 38.6 31 Y 11.5%	0.80 [0.32, 1.28]
l ² = 65%			
Pooled estimate: 7 – 3 37	452	271 100.0%	0.47 [0.20, 0.75]
P < 0.0007			
			12
Sensitivity analysis	Excluding serum (S): I ² = 59%, Z=2.4	1, <i>P</i> = 0.02	0.39 [0.07, 0.70]
	Excluding pediatric migraine (PM): I^2	= 65%, Z=3.56, <i>P</i> = 0.0004	0.58 [0.26, 0.89]
Enterally advectory 1.1.1			
בpisoαic migraine - ictal			
CSF	No studies published		
	Cases	Controls	SMD (95% CI)
Blood	N- Mean SD Age For MO	N- Mean SD Are Fem H Weight	Bandom-effects
Goadsby et al 1990	P 22 41 6 19.6 36 16 12	12 35.0 22.3 NB ND V 17.0%	0.31 [-0.39, 1.02]
Gallai et al 1995 (PM)	P 75 50 74 43,80 15.9 32 45	30 38.2 35.6 15.1 15 Y 49.7%	0.30 [-0.13, 0.72]
Fan et al. 2009 (PM)	P 25 236.8 216.5 NR NR NR	22 52.4 26.3 NB 9 N 23.3%	1.14 [0.52, 1.76]
Rodriguez et al. 2012	S 19 298,2 100.3 NR NR NR	23 37.1 38.5 31.1 22 Y 9.1%	3.50 [2.51, 4.50]
l ² = 92%			
Pooled estimate: Z = 5.15	141	87 100.0%	0.79 [0.49, 1.09]
<i>P</i> < 0.0001			
Sensitivity analysis	Excuding serum (S): I ² = 61%, Z=3	3.22, <i>P</i> = 0.001 0.1	52 [0.20, 0.83]
			-4 -2 0 2 4

Figure 4. Forest plot of CGRP concentrations in migraine patients and controls.

The squares represent effect sizes of the individual studies (size reflects the weight of the study) and the horizontal lines indicate the 95% confidence intervals (CI). The filled diamonds represent the overall effect size (horizontal width indicates the 95% CI). CGRP: calcitonin gene-related peptide; Age: mean age; Fem: number of females; MO: number of cases with migraine without aura; H: healthy controls; Y: yes; N: no; NR: not reported; P: plasma concentrations; S: serum concentrations. *Migraine state is not explicitly reported for this study, the interictal state was assumed. PM: paediatric migraine patients and paediatric controls. Additional information on the handling of missing data (e.g. calculations, assumptions) can be found in the supplement.

Chronic migraine														
	Cas	es				Co	ntrols						SMD (95% CI)	SMD (95% CI)
CSF	N=	Mean SD	Age	Fem	MO	N=	Mean	SD	Age	Fem	н	Weight	Fixed-effects	Ĩ
Sarchielli et al. 2001	20	39.3 5.9	43.6	15	NR	20	11.3	2.6	42.1	13	Ν	36.8%	6.02 [4.50, 7.54]	
Sarchielli et al. 2002	25	39.8 5.8	46.5	18	NR	20	11.7	2.7	44.9	13	Ν	43.3%	5.89 [4.48, 7.29]	
Sarchielli et al. 2007 (I)	20	46.7 4.6	38.4	16	NR	20	13.7	2.7	41.6	13	Ν	19.8%	8.58 [6.50, 10.65]	
$l^2 = 60\%$														
Pooled estimate: Z = 13.73	65					60						100%	6.47 [5.55, 7.39]	•
<i>P</i> < 0.0001														
	Cas	es				Co	ntrols						SMD (95% CI)	
Blood	N=	Mean SD	Age	Fem	MO	N=	Mean	SD	Age	Fem	Н	Weight	Unpooled	
Jang et al. 2011	P 33	41.1 21.5	43.7	21	NR	36	21.6	13.5	44.3	19	Y	NA	1.08 [0.58, 1.59]	+
Episodic migraine - Interic	tal													
CSF	No s	studies publ	ished											
	Cases												SMD (95% CI)	
Blood	N=	Mean SD	Age	Fem	MO	N=	Mean	SD	Age	Fem	н	Weight	Unpooled	
Blandini et al. 2006	P 60	26.3 57.2	35.8	41	33	57	23.8	27.0	34.5	30	Υ	NA	0.06 [-0.31, 0.42]	+
													+ + +	
													-10 -5	0 5 10
													Lower	Higher

Figure 5. Forest plot of NGF concentrations in migraine patients and controls.

The squares represent effect sizes of the individual studies (size reflects the weight of the study) and the horizontal lines indicate the 95% confidence intervals (CI). The filled diamonds represent the overall effect size (horizontal width indicates the 95% CI). NGF: nerve growth factor; Age: mean age; Fem: number of females; MO: number of cases with migraine without aura; H: healthy controls; Y: yes; N: no; NR: not reported; P: plasma concentrations. Additional information on the handling of missing data (e.g. calculations, assumptions) can be found in the supplement.

(SMD: -0.76, 95% CI: -1.17, -0.36) (38,63,64) from chronic migraine patients. Concentrations were also decreased in CSF (SMD: -1.12, 95% CI: -1.65, -0.58) (37,38,40) and blood (SMD: -0.40, 95% CI: -0.64, -0.16) (38,40,65–73) from interictal patients. Pooled estimates remained similar in sensitivity analysis. One blood study was excluded from meta-analysis because the assay that was used had a very high cross-reactivity with β -lipotropin (28). In ictal migraineurs β -EP concentrations were decreased in CSF (SMD: -1.39, 95% CI: -2.40, -0.39) (40) and blood (SMD: -1.44, 95% CI: -2.11, -0.77) (40,66).

CGRP (Figure 4)

CGRP concentrations were increased in CSF (SMD: 3.80, 95% CI: 3.19, 4.41) (18,42,43) and blood (SMD: 0.70, 95% CI: 0.45, 0.95) (74–77) from chronic migraine patients, and in blood from interictal (SMD: 0.47, 95% CI: 0.20, 0.75) (74,76–83) and ictal (SMD: 0.79, 95% CI: 0.49, 1.09) (78,82–84) episodic migraineurs. Sensitivity analysis had small effects on pooled estimates. There are no studies on CGRP concentrations in CSF from episodic migraine patients.

NGF (Figure 5)

Concentrations of NGF were increased in CSF (SMD: 6.47, 95% CI: 5.55, 7.39) (20,21,42) and blood (SMD: 1.08, 95% CI: 0.58, 1.59) (75) from chronic migraine patients. Blood concentrations were not significantly different in interictal patients (SMD: 0.06, 95% CI: – 0.31, 0.42) (85). There are no studies published on ictal concentrations (CSF and blood) and interictal concentrations in CSF.

Discussion

We conducted a systematic review and meta-analysis of biochemical measurements in CSF from chronic and episodic migraineurs. Meta-analysis showed increased concentrations of glutamate and CGRP and decreased concentrations of β -EP in CSF. These changes are also present in blood – a more accessible body fluid. Concentrations of NGF were increased in CSF from chronic migraine patients but blood data were limited.

Increases in glutamate and CGRP are in agreement with theories on pathophysiological mechanisms for migraine (4,5). Glutamate is the principal excitatory neurotransmitter within the central nervous system and has been linked to neuronal hyperexcitability in migraine (86). Glutamate has been implicated in the onset and generation of cortical spreading depression (CSD), which is believed to be the underlying cause of migraine aura. Increased synaptic glutamate concentrations lower the threshold for CSD (5). CGRP has been implicated as a mediator which activates and sensitises peripheral meningeal nociceptors causing migraine headache (4). Trigeminal fibres surrounding meningeal vessels release CGRP and other neuropeptides, and there is increasing evidence that CSD can initiate this release in animal experiments (5). Another rat model showed that CGRP released by primary trigeminal afferents impacts both CSF and blood concentrations and that the contribution of non-trigeminal structures to CSF concentrations is only minor (87).

In CSF from chronic migraine patients, NGF is increased with glutamate and CGRP. NGF is not only a well-known growth factor, but, following tissue injury, also an inducer of hyperalgesia via different peripheral mechanisms including mast cell degranulation (88). After local injury or inflammation both peripheral (reactivated Schwann cells, non-neural cells) and central (neurons, astrocytes, microglia) sources upregulate NGF expression (89). In contrast to CGRP, their relative contributions to CSF composition are still unknown. Additionally, NGF can upregulate CGRP expression in sensory and motor neurons (90,91). In one included CSF study NGF and CGRP concentrations were indeed positively correlated (42). Furthermore, by upregulating synthesis of brainderived neurotrophic factor (BDNF), NGF can enhance synaptic transmission via N-methyl-Daspartate receptors (92,93). Primarily the latter is hypothesised to contribute to chronic sensitisation of central neurons (i.e. in the nucleus trigeminus) (19-22). It is believed that this process also occurs in other chronic pain disorders such as fibromyalgia, where increased CSF concentrations of NGF, BDNF and glutamate have been found (21). This indicates that the observed changes are possibly not specific for migraine and instead reflect exposure to chronic pain.

 β -EP concentrations are decreased in CSF and blood both from interictal patients and chronic migraine patients. Low β -EP concentrations have been hypothesised to reflect low analgesic activity in individuals. However, recent evidence suggests chronic pain patients with low β -EP concentrations have stronger analgesic activity when in pain through rapid upregulation of β -EP (94). In analgesic research with migraine patients β -EP could be a useful marker to study in more detail.

Study strengths and limitations

The main strength of this study is the systematic approach to the identification, quality assessment and analysis of published data. However, our findings should be interpreted in the light of the limitations of the included evidence.

We found considerable clinical and methodological heterogeneity across studies. Statistical heterogeneity was also observed in meta-analysis of glutamate, β-EP and CGRP, but importantly all studies showed an effect in the same direction. Nonetheless, diversity in migraine patients was present due to differences in migraine frequency, timing of measurements and diagnostic criteria. Less diversity was present in CSF studies on CGRP and NGF, since the studies were performed at the same headache centre (new participants with approximately similar clinical characteristics (age, gender, disease history, headache frequency and medication overuse) were recruited for each study; confirmed with original investigators). Furthermore, migraine patients were not always representative for the diagnosed migraine type because samples were taken for diagnostic purposes (i.e. other neurological disorders were suspected). Diversity in controls seemed primarily related to the availability of samples as well. Control cohorts often consisted of nonhealthy controls in whom samples were collected for other diagnostic purposes than the migraine patients; this is especially the case for CSF studies where collection in healthy individuals is often not possible.

Additionally we found that the quality of reporting was inconsistent. Studies particularly failed to specify the validation, sensitivity and monitoring of applied measurement technique. Additionally, group comparisons were not always clearly reported and applied statistical analysis was frequently not well explained. Therefore, despite our attempts to contact corresponding authors, we were not able to retrieve all required data and had to apply published methods to calculate or estimate these data (14).

Publication bias is probably a major issue in the reporting of biomarker studies, because negative findings are less likely to get published (95). However, we did not generate funnel plots to assess any publication bias because the power of this strategy is low with the relative small number of studies per compound.

Recommendations

Future research should further clarify the pathophysiological relevance of the altered glutamate, β -EP, CGRP and NGF concentrations in migraine. For better understanding of involved biochemical processes, and for potential application as diagnostic biomarkers, it is also important to know whether concentrations are altered in all migraine types and whether similar changes are present in other headache disorders and chronic pain disorders.

Article highlights

- This is the first meta-analysis of biochemical measurements in cerebrospinal fluid (CSF) and blood from chronic and episodic migraine patients.
- A total of 62 unique compounds have been measured in CSF from migraine patients.
- Glutamate, calcitonin gene-related peptide and nerve growth factor (NGF) concentrations are increased and β-endorphin concentrations are decreased in CSF from migraine patients.
- These changes are also present in blood, with the exception of NGF.
- The presented data identify clear biomarker targets for future pathophysiological or diagnostic studies on migraine.

Funding

This work was supported by the Netherlands Organisation for Scientific Research (VICI grant 918.56.601 to M.D.F. and VIDI grant 917.11.319 to G.M.T.) and the European Union's Seventh Framework programme (EUROHEADPAIN, grant agreement no. 602633).

Declaration of conflicting interests

R.M. van Dongen, M. Noga, O.M. Dekkers, T. Hankemeier and A.M.J.M. van de Maagdenberg declare no potential conflicts of interest with respect to the research, authorship, and/ or publication of this article. R. Zielman disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: support for conference visits from Menarini and Allergan. G.M. Terwindt disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: independent support from NWO, ZonMW, the National Institutes of Health (NIH), the European Community, and the Dutch Heart Foundation. M.D. Ferrari disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: grants and consultancy or industry support from Medtronic, and independent support from NWO, ZonMW, NIH, the European Community, and the Dutch Heart Foundation.

Acknowledgements

We would like to thank the collaborating corresponding authors who were so kind to answer our questions and the research librarians who aided with formulating the search string.

Author contributions are as follows: R.M. van Dongen and R. Zielman were responsible for study design, data acquisition, data analysis, data interpretation and drafting/revising manuscript content. M. Noga was responsible for study assessment (biochemical measurements). O.M. Dekkers was responsible for data analysis (meta-analysis), data interpretation and revising manuscript content. T. Hankemeier, G.M. Terwindt, A.M.J.M. van den Maagdenberg and M.D. Ferrari were responsible for data interpretation and revising manuscript content.

References

- Robbins MS and Lipton RB. The epidemiology of primary headache disorders. *Semin Neurol* 2010; 30: 107–119.
- Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990– 2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015; 386: 743–800.
- 3. Tfelt-Hansen PC and Koehler PJ. One hundred years of migraine research: Major clinical and scientific observations from 1910 to 2010. *Headache* 2011; 51: 752–778.
- Ho TW, Edvinsson L and Goadsby PJ. CGRP and its receptors provide new insights into migraine pathophysiology. *Nat Rev Neurol* 2010; 6: 573–582.
- Pietrobon D and Moskowitz M. Pathophysiology of migraine. Annu Rev Physiol 2013; 75: 1–27.
- Pepe MS, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001; 93: 1054–1061.
- Mischak H, Ioannidis JP, Argiles A, et al. Implementation of proteomic biomarkers: Making it work. *Eur J Clin Invest* 2012; 42: 1027–1036.
- Rosén C, Hansson O, Blennow K, et al. Fluid biomarkers in Alzheimer's disease – current concepts. *Mol Neurodegener* 2013; 8: 20.
- Ripley B, Overeem S, Fujiki N, et al. CSF hypocretin/ orexin levels in narcolepsy and other neurological conditions. *Neurology* 2001; 57: 2253–2258.
- Kang J, Irwin D, Chen-Plotkin A, et al. Association of cerebrospinal fluid β-amyloid 1-42, T-tau, P-tau181, and α-synuclein levels with clinical features of drug-naive patients with early Parkinson disease. JAMA Neurol 2013; 70: 1277–1287.
- Brown PD, Davies SL, Speake T, et al. Molecular mechanisms of cerebrospinal fluid production. *Neuroscience* 2012; 129: 957–970.
- Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ* 2009; 339: b2535.

- Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses, http://www.ohri.ca/ programs/clinical_epidemiology/oxford.asp (2006, accessed 17 December 2013).
- 14. The Cochrane Collaboration. *The Cochrane handbook for systematic reviews of interventions*. Version 5, 2011.
- Headache Classification Committee of the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. *Cephalalgia* 1988; 8: 1–96.
- Siberstein SD, Lipton RB, Solomon S, et al. Classification of daily and near-daily headaches: Proposed revisions to the IHS criteria. *Headache* 1994; 34: 1–7.
- Silberstein SD, Olesen J, Bousser MG, et al. The International Classification of Headache Disorders, 2nd Edition (ICHD-II) – revision of criteria for 8.2 Medicationoveruse headache. *Cephalalgia* 2005; 25: 460–465.
- Gallai V, Alberti A, Gallai B, et al. Glutamate and nitric oxide pathway in chronic daily headache: Evidence from cerebrospinal fluid. *Cephalalgia* 2003; 23: 166–174.
- Peres MFP, Zukerman E, Senne Soares C, et al. Cerebrospinal fluid glutamate levels in chronic migraine. *Cephalalgia* 2004; 24: 735–739.
- Sarchielli P, Alberti A, Gallai B, et al. Brain-derived neurotrophic factor in cerebrospinal fluid of patients with chronic daily headache: Relationship with nerve growth factor and glutamate levels. *J Headache Pain* 2002; 3: 129–135.
- Sarchielli P, Mancini ML, Floridi A, et al. Increased levels of neurotrophins are not specific for chronic migraine: Evidence from primary fibromyalgia syndrome. *J Pain* 2007; 8: 737–745.
- Vieira D, Naffah-Mazzacoratti MDG, Zukerman E, et al. Glutamate levels in cerebrospinal fluid and triptans overuse in chronic migraine. *Headache* 2007; 47: 842–847.
- 23. Rothrock JF, Mar KR, Yaksh TL, et al. Cerebrospinal fluid analyses in migraine patients and controls. *Cephalalgia* 1995; 15: 489–493.
- Martínez F, Castillo J and Rodríguez J. Neuroexcitatory amino acid levels in plasma and cerebrospinal fluid during migraine attacks. *Cephalalgia* 1993; 13: 90–93.
- Kangasniemi P, Sonninen V and Rinne UK. Excretion of free and conjugated 5-HIAA and VMA in urine and concentration of 5-HIAA and HVA in CSF during migraine attacks and free intervals. *Headache* 1972; 12: 62–65.
- 26. Poloni M, Nappi G, Arrigo A, et al. Cerebrospinal fluid 5-hydroxyindoleacetic acid level in migrainous patients during spontaneous attacks, during headache-free periods and following treatment with L-tryptophan. *Experentia* 1974; 30: 640–641.
- Kovács K, Bors L, Tóthfalusi L, et al. Cerebrospinal fluid (CSF) investigations in migraine. *Cephalalgia* 1989; 9: 53–57.
- Anselmi B, Elisabetta B, Casacci F, et al. Endogenous opioids in cerebrospinal fluid and blood in idiopathic headache sufferers. *Headache* 1980; 20: 294–299.
- Salmon S and Bonciani M. A putative 5-HT central feedback in migraine and cluster headache attacks. *Adv Neurol* 1982; 33: 265–274.

- Facchinetti F, Sances G and Martignoni E. Evidence of alpha- N-acetyl β-endorphin in human cerebrospinal fluid. *Brain Res* 1992; 586: 1–5.
- 31. Castillo J, Martinez F, Suarez C, et al. Cerebrospinal fluid tyrosine and 3, 4-dihydroxyphenylacetic acid levels in migraine patients. *Cephalalgia* 1996; 16: 56–61.
- Martínez F, Castillo J, Pardo J, et al. Catecholamine levels in plasma and CSF in migraine. J Neurol Neurosurg Psychiatry 1993; 56: 1119–1121.
- Vieira DSS, Naffah-Mazacoratti MG, Zukerman E, et al. Cerebrospinal fluid GABA levels in chronic migraine with and without depression. *Brain Res* 2006; 1090: 197–201.
- Welch K, Chabi E and Bartosh K. Cerebrospinal fluid gamma aminobutyric acid levels in migraine. *Br Med J* 1975; 3: 516–517.
- 35. Welch K, Chabi E and Nell J. Biochemical comparison of migraine and stroke. *Headache* 1976; 16: 160–167.
- Kunkle EC and Durham N. Acetylcholine in the mechanism of headaches of migraine type. AMA Arch Neurol Psychiatry 1959; 81: 135–141.
- Genazzani A, Nappi G, Facchinetti F, et al. Progressive impairment of CSF β-EP levels in migraine sufferers. *Pain* 1984; 18: 127–133.
- Nappi G, Facchinetti F, Martignoni E, et al. Plasma and CSF endorphin levels in primary and symptomatic headaches. *Headache* 1985; 25: 141–144.
- Nappi G, Facchinetti F, Martignoni E, et al. CSF β-EP in headache and depression. *Cephalalgia* 1985; 5: 99–101.
- Vécsei L, Widerlöv E, Ekman R, et al. Suboccipital cerebrospinal fluid and plasma concentrations of somatostatin, neuropeptide Y and beta-endorphin in patients with common migraine. *Neuropeptides* 1992; 22: 111–116.
- Simonnet G, Taquet H, Floras P, et al. Simultaneous determination of radio-immunoassayable methionineenkephalin and radioreceptor-active opiate peptides in CSF of chronic pain suffering and non suffering patients. *Neuropeptides* 1986; 7: 229–240.
- 42. Sarchielli P, Alberti A, Floridi A, et al. Levels of nerve growth factor in cerebrospinal fluid of chronic daily headache patients. *Neurology* 2001; 57: 132–134.
- Sarchielli P, Pini LA, Coppola F, et al. Endocannabinoids in chronic migraine: CSF findings suggest a system failure. *Neuropsychopharmacology* 2007; 32: 1384–1390.
- 44. Valenzuela RF, Donoso MV, Mellado PA, et al. Migraine, but not subarachnoid hemorrhage, is associated with differentially increased NPY-like immunoreactivity in the CSF. J Neurol Sci 2000; 173: 140–146.
- Sarchielli P, Alberti A, Candeliere A, et al. Glial cell linederived neurotrophic factor and somatostatin levels in cerebrospinal fluid of patients affected by chronic migraine and fibromyalgia. *Cephalalgia* 2006; 26: 409–415.
- 46. Sarchielli P, Rainero I, Coppola F, et al. Involvement of corticotrophin-releasing factor and orexin-A in chronic migraine and medication-overuse headache: findings from cerebrospinal fluid. *Cephalalgia* 2008; 28: 714–722.
- 47. Rozen T and Swidan SZ. Elevation of CSF tumor necrosis factor alpha levels in new daily persistent headache

and treatment refractory chronic migraine. *Headache* 2007; 47: 1050–1055.

- Bø SH, Davidsen EM, Gulbrandsen P, et al. Cerebrospinal fluid cytokine levels in migraine, tension-type headache and cervicogenic headache. *Cephalalgia* 2009; 29: 365–372.
- Harrington MG, Fonteh AN, Cowan RP, et al. Cerebrospinal fluid sodium increases in migraine. *Headache* 2006; 46: 1128–1135.
- 50. Martinez F, Castillo J, Leira R, et al. Taurine levels in plasma and cerebrospinal fluid in migraine patients. *Headache* 1993; 33: 324–327.
- Elwan O, Abdella M, El Bayad A, et al. Hormonal changes in headache patients. J Neurol Sci 1991; 106: 75–81.
- 52. Isobe C and Terayama Y. A remarkable increase in total homocysteine concentrations in the CSF of migraine patients with aura. *Headache* 2010; 50: 1561–1569.
- Casmiro M, Scarpa E, Cortelli P, et al. Cerebrospinal fluid and serum neuron-specific enolase in acute benign headache. *Cephalalgia* 2008; 28: 506–509.
- Fonteh AN, Chung R, Sharma TL, et al. Cerebrospinal fluid phospholipase C activity increases in migraine. *Cephalalgia* 2011; 31: 456–462.
- 55. Fonteh AN, Pogoda JM, Chung R, et al. Phospholipase C activity increases in cerebrospinal fluid from migraineurs in proportion to the number of comorbid conditions: a case-control study. J Headache Pain 2013; 14: 60.
- Ferrari MD, Odink J, Bos KD, et al. Neuroexcitatory plasma amino acids are elevated in migraine. *Neurology* 1990; 40: 1582–1586.
- Cananzi A, D'andrea G and Perini F. Platelet and plasma levels of glutamate and glutamine in migraine with and without aura. *Cephalalgia* 1995; 15: 132–135.
- D'Eufemia P, Finocchiaro R, Lendvai D, et al. Erythrocyte and plasma levels of glutamate and aspartate in children affected by migraine. *Cephalalgia* 1997; 17: 652–657.
- Alam Z, Coombes N, Waring RH, et al. Plasma levels of neuroexcitatory amino acids in patients with migraine or tension headache. *J Neurol Sci* 1998; 156: 102–106.
- Vaccaro M, Riva C, Tremolizzo L, et al. Platelet glutamate uptake and release in migraine with and without aura. *Cephalalgia* 2007; 27: 35–40.
- 61. Ferrari A, Spaccapelo L, Pinetti D, et al. Effective prophylactic treatments of migraine lower plasma glutamate levels. *Cephalalgia* 2009; 29: 423–429.
- Campos F, Sobrino T, Pérez-Mato M, et al. Glutamate oxaloacetate transaminase: a new key in the dysregulation of glutamate in migraine patients. *Cephalalgia* 2013; 33: 1148–1154.
- Martignoni E, Facchinetti F, Rossi F, et al. Neuroendocrine evidence of deranged noradrenergic activity in chronic migraine. *Psychoneuroendocrinology* 1989; 14: 357–363.
- Misra UK, Kalita J, Tripathi GM, et al. Is β endorphin related to migraine headache and its relief? *Cephalalgia* 2013; 33: 316–322.
- 65. Facchinetti F, Nappi G, Savoldi F, et al. Primary headaches: reduced circulating beta-lipotropin and betaendorphin levels with impaired reactivity to acupuncture. *Cephalalgia* 1981; 1: 195–201.

- Baldi E, Salmon S, Anselmi B, et al. Intermittent hypoendorphinaemia in migraine attack. *Cephalalgia* 1982; 2: 77–81.
- Facchinetti F, D'Attoma G, Petraglia F, et al. Circadian variations of proopiocortin-related peptides in children with migraine. *Cephalalgia* 1983; Suppl 1: 7–10.
- 68. Fettes I, Gawel M, Kuzniak S, et al. Endorphin levels in headache syndromes. *Headache* 1985; 25: 37–39.
- Facchinetti F, Moglia A, Bonuccelli U, et al. Pattern of plasma opioids in menstrually-related migraine and epilepsy. *Funct Neurol* 1986; 1: 415–419.
- Awaki E, Takeshima T and Takahashi K. A neuroendocrinological study in female migraineurs: prolactin and thyroid stimulating hormone responses. *Cephalalgia* 1989; 9: 187–193.
- Facchinetti F, Martignoni E and Nappi G. Premenstrual failure of α-adrenergic stimulation on hypothalamuspituitary responses in menstrual migraine. *Pyschosomatc Med* 1989; 51: 550–558.
- Vd Helm-Hylkema H, Orlebeke JF, Enting LA, et al. Effects of behaviour therapy on migraine and plasma beta-endorphin in young migraine patients. *Psychoneuroendocrinology* 1990; 15: 39–45.
- Battistella P, Bordin A, Cernetti R, et al. Beta-endorphin in plasma and monocytes in juvenile headache. *Headache* 1996; 36: 91–94.
- Gupta R, Ahmed T, Banerjee B, et al. Plasma calcitonin gene-related peptide concentration is comparable to control group among migraineurs and tension type headache subjects during inter-ictal period. *J Headache Pain* 2009; 10: 161–166.
- Jang M-U, Park J-W, Kho H-S, et al. Plasma and saliva levels of nerve growth factor and neuropeptides in chronic migraine patients. *Oral Dis* 2011; 17: 187–193.
- Cernuda-Morollón E, Larrosa D, Ramón C, et al. Interictal increase of CGRP levels in peripheral blood as a biomarker for chronic migraine. *Neurology* 2013; 81: 1191–1196.
- Oterino A, Toriello M, Palacio E, et al. Analysis of endothelial precursor cells in chronic migraine: a casecontrol study. *Cephalalgia* 2013; 33: 236–244.
- Gallai V, Sarchielli P, Floridi A, et al. Vasoactive peptide levels in the plasma of young migraine patients with and without aura assessed both interictally and ictally. *Cephalalgia* 1995; 15: 384–390.
- Ashina M, Bendtsen L, Jensen R, et al. Evidence for increased plasma levels of calcitonin gene-related peptide in migraine outside of attacks. *Pain* 2000; 86: 133–138.
- 80. Juhasz G, Zsombok T, Laszik A, et al. Despite the general correlation of the serotonin transporter gene regulatory region polymorphism (5-HTTLPR) and platelet serotonin concentration, lower platelet serotonin concentration in migraine patients is independent of the 5-HTTLPR variants. *Neurosci Lett* 2003; 350: 56–60.
- Fusayasu E, Kowa H, Takeshima T, et al. Increased plasma substance P and CGRP levels, and high ACE activity in migraineurs during headache-free periods. *Pain* 2007; 128: 209–214.
- Fan P-C, Kuo P-H, Chang S-H, et al. Plasma calcitonin gene-related peptide in diagnosing and predicting paediatric migraine. *Cephalalgia* 2009; 29: 883–890.

- 83. Rodríguez-Osorio X, Sobrino T, Brea D, et al. Endothelial progenitor cells: a new key for endothelial dysfunc-
- tion in migraine. *Neurology* 2012; 79: 474–479.
 84. Goadsby PJ, Edvinsson L and Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol* 1990; 28: 183–187.
- Blandini F, Rinaldi L, Tassorelli C, et al. Peripheral levels of BDNF and NGF in primary headaches. *Cephalalgia* 2006; 26: 136–142.
- 86. Ramadan NM. Glutamate and migraine: From Ikeda to the 21st century. *Cephalalgia* 2013; 0: 1–4.
- Hoffman J, Sascha W, Neeb L, et al. Primary trigeminal afferents are the main source for stimulus-induced CGRP release into jugular vein blood and CSF. *Cephalalgia* 2012; 32: 659–667.
- Lewin GR, Rueff A and Mendell LM. Peripheral and central mechanisms of NGF-induced hyperalgesia. *Eur J Neurosci* 1994; 6: 1903–1912.
- Sofroniew MV, Howe CL and Mobley WC. Nerve growth factor signaling, neuroprotection, and neural repair. *Ann Rev Neurosci* 2001; 24: 1271–1281.
- 90. Ramer M, Bradbury E, Michael G, et al. Glial cell linederived neurotrophic factor increases calcitonin gene-

related peptide immunoreactivity in sensory and motoneurons in vivo. *Eur J Neurosci* 2003; 18: 2713–2721.

- Price TJ, Louria MD, Candelario-Soto D, et al. Treatment of trigeminal ganglion neurons in vitro with NGF, GDNF or BDNF: effects on neuronal survival, neurochemical properties and TRPV1-mediated neuropeptide secretion. *BMC Neurosci* 2005; 6.
- 92. Lin S, Wu K, Levine ES, et al. BDNF acutely increases tyrosine phosphorylation of the NMDA receptor subunit 2B in cortical and hippocampal postsynaptic densities. *Mol Brain Res* 1998; 55: 20–27.
- Kerr BJ, Bradbury EJ, Bennett DLH, et al. Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord. J Neurosci 1999; 19: 5138–5148.
- Bruehl S, Burns J, Chung O, et al. What do plasma betaendorphin levels reveal about endogenous opioid analgesic function? *Eur J Pain* 2012; 16: 370–380.
- Tzoulaki I, Siontis K, Evangelou E, et al. Bias in associations of emerging biomarkers with cardiovascular disease. JAMA Intern Med 2013; 173: 664–671.