Serotonin and Its Metabolite; 5-Hydroxyindoleacetic Acid in Neurophysiology of Headache

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Received 2 August, 2002 ; Accepted 12 March, 2003

Summary **Although some trace amines have clearly defined roles as neurotransmitters in human, their exact role in the neurophysiology of headache remains unclear. In this study, plasma levels of one trace biogenic amine; serotonin (5-HT) and its metabolite; 5-hydroxyindoleacetic acid (5-HIAA) were assayed by high performance liquid chromatography with electrochemical detection in 50 subjects suffering from chronic headache due to various aetiologies including migraine. Moreover, 30 normal healthy subjects were also included in the study as control group. Results of the present work demonstrated significant decrease in plasma levels of both 5-HT and 5-HIAA in patients with chronic headache as compared with control subjects. Furthermore, 5-HT and 5-HIAA levels were not found to be correlated with the duration of headache, age and sex of the patient groups studied. Moreover, the two parameters exhibited significant decrease in subjects with migraine as compared with subjects with chronic headache due to causes other than migraine. This finding points to the major role played by 5-HT deficiency in the pathophysiology of headache in general and migraine in particular. The identifications of the exact pathophysiology of headache and migraine lead to the development of novel therapeutics for subjects with migraine as well as non migranous chronic headache.**

Key Words: **migraine, chronic headache, serotonin, 5-hydroxyindoleacetic acid**

Introduction

Migraine remains an extremely common problem worldwide in frequency, severity and level of disability. Migraine attacks occur in 18% of women and 6% of men. Migraine headache was described as having 4 distinct parts: First is the initiation or premonitoring symptoms, often hours to days ahead of more specific migraine symptoms. Second is the attack or the aura. Third is the pain and associated symptoms. Finally is the resolution phase. Most patients have warning of an impending attack in the initiation stage of fatigue, irritability, difficulty of thinking, or difficulty of concentration as well as other symptoms [*1*].

The migraine process involves a combination of events, including release of inflammatory neurokinins (substance P), and free radicals formation (nitric oxide) augmented by complex mechanisms of afferent and efferent trigeminal vascular events further mediated by central modulation. This process has been increasingly understood as the pathogenesis of migraine headache. This understanding is leading to better and more efficient specific treatment options [*2*].

The aim of this work is to study levels of 5-HT and its metabolite 5-HIAA in plasma of patients with chronic headache of different causes including migraine, and to study the relations of plasma levels of these two parameters to the duration of headache, age and sex of the patient groups studied.

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Materials and Methods

Subjects

Fifty patients with chronic headache were selected from Neurology Department, Kasr El-Aini Hospital, Faculty of Medicine, Cairo University. They constituted the subjects of this work. These patients were subdivided into two subgroups: first subgroup (Group I) included 25 subjects with migraine with or without aura, their age ranged from 11–62 years. They included 13 females and 12 males. Fifteen migranous subjects had headache less than 6 months and 10 subjects had headache for more than 6 months. The second subgroup (Group II) included 25 subjects with chronic headache due to causes other than migraine, their age ranged from 15–54 years, they included 14 males and 11 females, 4 subjects had headache less than 6 months and 21 subjects had headache for more than 6 months. This group included 3 subjects with cluster headache, 5 subjects with hypertensive headache, 6 subjects with headache of increased intracranial tension, 7 subjects with episodic tension headache and 4 subjects with chronic tension headache. Furthermore, 30 healthy subjects were also included in the study as control group (Group III), their age ranged from 17–55 years, they included 15 males and 15 females.

Evaluated parameters

The following parameters were evaluated for all subjects.

1. Full medical history and thorough neurological examination.

2. Plasma 5-HT and 5-HIAA levels were assayed by high performance liquid chromatography (HPLC) with electrochemical detection (Waters). Chromatographic separations were performed using 3-μm BAS Phase II ODS analytical column (100×3.2 mm I.D.) preceded by 7-μm BAS Phase II ODS pre-column (15×3.2 mm I.D.). The method involved extraction of 100 μl plasma samples with 500 μl perchloric acid containing 0.01% cysteine as antioxidant, 500 pg/100 μl isoprenaline as internal standard for 5-HT and 500 pg/100 μl deoxyepinephrine as internal standard for 5-HIAA. The mobile phase consisted of 0.1 M monochloroacetic acid, 0.65 mM sodium octyl sulphate, 0.5 mM EDTA in milli-Q water and the pH was adjusted to 3.05 with 6 M NaOH. All chemicals were HPLC grade (SIGMA, St. Louis, MO, USA). Following filtration through GV 0.22 μm filter (Waters) acetonitrile was added to a final volume concentration of 2.9%. The mobile phase was degassed through vacuum degasser (BAS-LC 26). The chromatographic separation was performed isocratically at a flow rate of 1.0 ml/min. The detector potential was at 0.45 volt *vs.* silver electrode Ag/AgCl. Injection volume was 50 μl.

Statistical analysis

Statistical analysis was done by using statistical package software (SPSS) and Microsoft Excell, 2000. Results were described as mean±SD, 95% tile or percentile is expressed as confidence interval or (CI), which is 2.5% outlayer from both sides of the range. Differences and regressions were regarded as statistically significant at $p<0.05$.

Results

Plasma 5-HT and 5-HIAA levels were significantly decreased in both of migranous and non migranous subjects with chronic headache as compared with control group (Table 1). Furthermore, migranous subjects exhibited more significant decrease in 5-HT and 5-HIAA levels as compared with non migranous subjects (Table 1). There were no significant correlation between levels of either 5-HT or 5-HIAA on one hand and duration of headache, age or sex of patients on the other hand (Tables

Table 1. 5-Hydroxyindoleacetic acid (ng/ml) and serotonin (ng/ml) levels in all chronic headache subjects and control subjects.

	5-HIAA (ng/ml)	*p1*	*p2*	5-HT (ng/ml)	*p1*	*p2*
Group I (n=25)	17.46±21.44	<0.0001	...	14.2±4	<0.0001	...
Group II (n=25)	68±13.98	<0.02	<0.0001	36.6±24.82	<0.0001	<0.05
Groups I and II (n=50)	42.73±31.34	<0.0001	...	25.4±20.73	<0.0001	...
Group III (n=30)	245±159	...	...	154±57	...	...

Results are expressed as mean±SD. *p1*, Comparison between each patient group *vs.* control group; *p2*, comparison between migranous subjects *vs.* non migranous subjects.

Table 2. Correlations between serotonin or 5-hydroxyindoleacetic on one hand and the duration of headache, age or sex of the patients on the other hand in 25 chronic headache subjects with migraine.

	5-HT		5-HIAA	
	r	*p*	*r*	*p*
Age	0.212	N.S.	0.216	N.S.
Sex	0.243	N.S.	0.205	N.S.
Duration of headache	0.261	N.S.	0.266	N.S.

Table 3. Correlations between serotonin or 5-hydroxyindoleacetic acid on one hand and the duration of headache, age or sex of the patients on the other hand in 25 chronic headache subjects due to causes other than migraine.

	5-HT		5-HIAA	
	r	*p*	*r*	*p*
Age	0.217	N.S.	0.226	N.S.
Sex	0.239	N.S.	0.245	N.S.
Duration of headache	0.259	N.S.	0.261	N.S.

2 and 3).

Statistical analysis of 5-HT in the following groups

Control group
- Sample variance=10.4
- Coefficient of variation=2.08
- Population variance=9.89
- Coefficient of variation=2.03
- Normal distribution goodness of fit test
- The hypothesis that the population is normal of mean 154 and STD.
- DEV. of 57 can not be rejected at the 95% confidence level or percentile.
- CHI SQUARE=6.2, DF=5, p=0.05

All headache cases
- Sample variance=2.5
- Coefficient of variation=5.8
- Population variance=2.4
- Coefficient of variation=5.6
- Normal distribution goodness of fit test
- The hypothesis that the population is normal of mean 25 and STD.
- DEV. of 21 can not be rejected at the 95% confidence level or percentile.
- CHI SQUARE=9.6, DF=5, p=0.05

Migraine cases
- Sample variance=3.2
- Coefficient of variation=6.2
- Population variance=3.3
- Coefficient of variation=5.6
- Normal distribution goodness of fit test
- The hypothesis that the population is normal of mean 14 and STD.
- DEV. of 4 can not be rejected at the 95% confidence level or percentile.
- CHI SQUARE=5.4, DF=5, p=0.03

Non migraine cases
- Sample variance=6.03
- Coefficient of variation=6.13
- Population variance=5.39
- Coefficient of variation=6.2
- Normal distribution goodness of fit test
- The hypothesis that the population is normal of mean 37 and STD.
- DEV. of 25 can not be rejected at the 95% confidence level or percentile.
- CHI SQUARE=6.2, DF=5, p=0.04

Statistical analysis of 5-HIAA in the following groups

Control group
- Sample variance=19.8
- Coefficient of variation=12.38
- Population variance=19.32
- Coefficient of variation=22.58
- Normal distribution goodness of fit test
- The hypothesis that the population is normal of mean 245 and STD.
- DEV. of 159 can not be rejected at the 95% confidence level or percentile.
- CHI SQUARE=9.4, DF=5, p=0.01

All headache cases
- Sample variance=11.3
- Coefficient of variation=15.7
- Population variance=12.6
- Coefficient of variation=15.3
- Normal distribution goodness of fit test
- The hypothesis that the population is normal of mean 43 and STD.
- DEV. of 31 can not be rejected at the 95% confidence level or percentile.
- CHI SQUARE=9.1, DF=5, p=0.02

Migraine cases
- Sample variance=4.1
- Coefficient of variation=5.2
- Population variance=4.5
- Coefficient of variation=5.1
- Normal distribution goodness of fit test
- The hypothesis that the population is normal of

mean 17 and STD.
DEV. of 21 can not be rejected at the 95% confidence level or percentile.
CHI SQUARE=8.4, DF=5, p=0.03
Non migraine cases
Sample variance=16.11
Coefficient of variation=17.2
Population variance=14.3
Coefficient of variation=15.6
Normal distribution goodness of fit test
The hypothesis that the population is normal of mean 68 and STD.
DEV. of 14 can not be rejected at the 95% confidence level or percentile.
CHI SQUARE=9.1, DF=5, p=0.02

Non parametric statistical tests reveiled the following results.

Wilcoxon rank test for two groups

1. Control and all headache subjects
   Variable tested 5-hydroxyindoleacetic acid
   Sum of ranks, control=1,965
   Sum of ranks, all headache subjects=1,275
   Z=7.454, Prob=4.537 E-14

   Variable tested 5-hydroxytryptamine
   Sum of ranks, control=1,973
   Sum of ranks, all headache subjects=1,291
   Z=7.334, Prob=4.117 E-12
2. Control and migraine headache subjects
   Variable tested 5-hydroxyindoleacetic acid
   Sum of ranks, control=1,215
   Sum of ranks, migraine headache=325
   Z=6.339, Prob=1.158 E-10

   Variable tested 5-hydroxytryptamine
   Sum of ranks, control=1,213
   Sum of ranks, all headache subjects=339
   Z=6.231, Prob=1.117 E-12
3. Control and non migraine headache subjects
   Variable tested 5-hydroxyindoleacetic acid
   Sum of ranks, control=348.5
   Sum of ranks, non migraine headache=926.5
   Z=5.607, Prob=1.027 E-08

   Variable tested 5-hydroxytryptamine
   Sum of ranks, control=351.1
   Sum of ranks, all headache subjects=939
   Z=6.131, Prob=1.017 E-10

Discussion

Pathophysiology of migraine headache has attracted attention of many investigators. 5-HT metabolism is involved in the pathophysiology of migraine. Evers *et al.* [*1*] reported that 5-HT content in the platelet dense bodies decreased significantly during the migraine attack. Sarachielli *et al.* [*3*] stated that in patients with chronic headache, there is a depletion of 5-HT in platelet dense bodies as well as up-regulation of 5-HT platelet receptors, which was correlated with chronicization of the headache. Furthermore, the authors stated that there is an increase in nitric oxide (NO) and cGMP production by platelets. They suggested a role of cGMP-Ca^{2+} mediated activation in the 5-HT depletion by platelets. A similar depletion in the central serotonergic pathways occurs in these patients.

Another hypothesis has been put by Goldstein *et al.* [*4*] and Munno *et al.* [*5*] who described migraine headache in a certain category of women with autoimmune problems. These women have autoantibodies to many hormones and neurotransmitters, and Munno *et al.* [*5*] recommended autoimmune testing for these women to determine whether the migraine headache is just one of the constellation of autoimmune symptoms that can also cause infertility, implantation failure and recurrent pregnancy losses.

Results of the present study demonstrated that there is a significant decrease in 5-HT and 5-HIAA plasma levels in patients with migraine and chronic headache due to other causes. The observed decrease in 5-HT and 5-HIAA was not found to be correlated with age, sex, or duration of headache. These results coincide with those obtained by Milovanovic *et al.* [*6*] who found that the urinary and plasma levels of 5-HT and 5-HIAA were significantly decreased in migraine as well as in tension type headache patients with more significant decrease in the catabolism of 5-HT during the attacks of either migraine or tension type headache as evidenced by the significantly reduced urinary values of 5-HIAA. The involvement of serotonin in migraine and tension type headache was not related to the presence of aura.

The neurons of the central nervous systems depend on a combination of electrical and chemical signals to do their work. A calcium channel is a type of a gate on the end of the neurons that is operated electrically. When an electrical impulse of sufficient voltage passes down a nerve cell, its calcium channel

opens and releases chemical messengers that affect neighboring cells. 5-HT and dopamine are some of these chemical messengers found in the brain [7].

Durham *et al.* [8] clarified the role of 5-HT in migraine at the molecular level. They studied the calcitonin gene-related peptide (*CGRP*) that encodes two biologically active peptides; The hormone calcitonin found in the thyroid C cells and the *CGRP* that is expressed in central neurons. *CGRP* acts to lower serum calcium levels and it is the most potent peptide vasodilator known and helps to maintain cardiovascular homeostasis [9]. High serum levels of *CGRP* are associated with several pathological conditions including migraine. Edvinsson and Goadsby [10] stated that subjects with spontaneous attacks of migraine show release of calcitonin gene-related peptide (CGRP) in parallel and continuously accompanying headache. Cluster headache patients have rapid release of CGRP during bouts. Elevated levels of CGRP have been implicated in neurogenic inflammation. Recently, a 5-HT type-1 (5-HT 1) antimigraine drug was shown to selectively reduce *CGRP* levels and alleviates pain [11]. 5-HT1 agonists inhibited *CGRP* mRNA levels and repressed promoter activity through cAMP-response element (CRE). 5-HT 1 receptor agonists caused a robust and sustained increase in intracellular calcium that is likely responsible for mediating repression of *CGRP* promoter. 5-HT1 has little or no inhibitory effects on adenyle cyclase activity and cAMP levels, but elevated calcium levels induced by 5-HT1 can inhibit cyclic AMP response elements binding proteins (CREB) by stimulating CREB phosphatase with subsequent inhibition of phosphorylation of CREB [12].

The newer antimigraine drugs are 5-HT 1 receptor agonists such as Naratripan (Amerge), Rizatripan (Maxalt), Sumatripan (Imitrex), and Zolmitripan (Zomig). Durham *et al.* [12] clarified the role of these drugs in migraine abortion at the molecular level. The authors stated that during migraine, *CGRP* levels are elevated but then returned to normal by 5-HT 1 receptor agonists [13]. *CGRP* gene transcription and peptide secretion are increased by cAMP, whereas, *CGRP* gene transcription and peptide secretion are inhibited by prolonged elevation of calcium levels [7]. In both cases, the cAMP- dependent and/or calcium dependent regulation of *CGRP* gene expression may be potential targets of 5-HT 1 agonists.

On the basis of these findings, it could be concluded that both chronic headache and migraine processes involve deficiency of 5-HT. Further studies should help to clarify the exact signaling mechanisms and efficiencies of serotonergic drugs on *CGRP* gene expression and peptide secretion.

Acknowledgments

This work was technically supported through Unit of Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University. This work was funded through the project entitled (Upgrading a Research and a Diagnostic Center for Diagnosis of Infectious Diseases in Egypt). The Ministry of International Cooperation, Cairo, Egypt.

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