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LETTER TO THE EDITOR

Plasma calcitonin gene-related peptide concentration is comparable to control group among migraineurs and tension-type headache subjects during interictal period: response to comments by Tfelt-Hansen and Ashina

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Dear Editor,

We thank Drs. Tfelt-Hansen and Ashina [1] for their interest in our article [2]. They have raised a valid point, namely that measurement units should be similar in the scientific literature. We assume that different units mentioned in previous studies were provided by the respective manufacturer of the kit, and we followed the same custom. However, in the interest of uniformity in the literature, as indicated by Drs. Tfelt-Hansen and Ashina, we provide revised results here (Table 1).

A concern was raised regarding the higher inter-ictal concentration of plasma CGRP in the cubital blood than reported in previous studies [3–7]. The available literature provides conflicting results regarding plasma CGRP concentration in migraineurs, both ictal as well as inter-ictal, and even when the blood was collected from different areas (external jugular/internal jugular and cubital) [2–9]. Hence, no conclusion can be reached regarding the baseline concentration of CGRP in plasma. The higher concentration reported in the present study than in previous studies can be explained by a number of methodological factors. First, the CGRP has a very short half life, barely 7 min, hence we made sure that plasma samples were frozen to −70°C within 5 min of drawing the blood [10, 11]. In a number of studies the sample was centrifuged for 15 min [6, 8, 12]. This could have resulted in lower levels. Secondly, it must be remembered that peptides are unstable molecules and they need to be preserved at −70°C. At least in one study samples were stored at –20°C until analysis, and this could have affected the results [13]. Moreover, the plasma level of CGRP follows a circadian pattern and shows troughs and peaks, but at different times according to different studies [14, 15]. We had drawn blood samples between 9 a.m. and 12 noon, when CGRP concentration is highest [14]. Furthermore, sampling methods and anticoagulants may affect the quantitative analysis with some peptides [16]. These factors could have affected the results of previous studies as they used different anticoagulants, e.g. heparin or aprotinin [6, 8, 13]. We used EDTA tubes to collect the blood on the manufacturer's recommendation. Lastly, the measurement of CGRP is influenced by a number of factors present in the plasma that may cross-react with it (Peninsula Lab Inc., LLC), hence we used the extraction-free kit. This further reduces the chances of error.

The sensitivity of the ELISA kit used in this study was 21 pmol/l and the range was 0–6596 pmol/l (Peninsula Lab Inc., LLC) with intra-assay variation of less than 5%.

Thus, the results of these kinds of studies warrant further studies in this area with better methodologies, until conclusive evidence is yielded.

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Table 1 Plasma CGRP levels (pmol/l) among diagnostic sub-groups of migraineurs

Diagnosis	*n*	Mean	Standard deviation	95% confidence interval for mean		Minimum	Maximum
				Lower limit	Upper limit		
MO	37	303.43	154.11	255.93	350.92	182.05	817.94
MOMA	7	271.76	36.93	237.46	306.06	208.44	308.70
MA	6	303.43	131.92	163.58	443.27	179.42	554.09

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