

DETERMINATION OF LEVELS OF PRECURSORS AND METABOLITES OF 5-HYDROXYTRYPTAMINE IN HUMAN CSF BY GC-NICIMS

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Five disease conditions were investigated to elucidate any irregularities in the biosynthesis and metabolism of 5HT, namely Myalgic Encephalomyelitis (ME—a condition characterised by excessive fatigue and muscle pain after a viral illness, often one of the Coxsackie viruses), Multiple Sclerosis (MS—an autoimmune disease in which the myelin sheaths of nerve fibres are destroyed), Muscle Wasting Disease (MWD—a group of conditions including Motor Neurone Disease and Myasthenia Gravis), Benign Intracranial Hypertension (BIH) and Spinal Injuries. For each condition the concentrations of 5HT, 5-hydroxyindole-3-acetic acid (5HIAA), L-tryptophan and L-5-hydroxytryptophan were determined in CSF. Further, the levels of N-acetyl-5HT (a metabolite of 5HT), melatonin (a biosynthetic product of N-acetyl-5HT) and 6-hydroxymelatonin (the major metabolite of melatonin) were also determined. The CSF was obtained from a lumbar puncture between the L₁ and L₂ vertebrae of the vertebral column. Analysis was performed by gas chromatography-negative ion chemical ionisation mass spectroscopy (GC-NICIMS) utilising selected ion monitoring for the pentafluoropropionyl spirocyclic derivatives of 5HT, N-acetyl-5HT, melatonin and 6-hydroxymelatonin or the trifluoroethanol-pentafluoropropionyl derivatives of 5HIAA, tryptophan and 5-hydroxytryptophan. The extraction and derivatisation of 5HT, N-acetyl-5HT, melatonin and 6-hydroxymelatonin of 200ul aliquots of CSF was performed by slight modification of the procedure outlined by Markey and Colburn (1981). 5HIAA, tryptophan and 5-hydroxytryptophan were extracted and derivatised by the method of Macfarlane et al (1990) using 50ul aliquots of CSF. Both procedures yielded derivatives with suitable diagnostic ions for analysis by GC-NICIMS. [²H₅]5HT, [²H₅]5HIAA and [²H₄]tryptophan were synthesised in our laboratory based on the methods of Matthews et al (1977) and Muskiet et al (1978). Calibration curves for these derivatives gave linear responses over the concentrations examined (r > 0.997). The levels of 5-hydroxytryptamine (n=9), 5-hydroxytryptophan (n=25), melatonin (n=9), N-acetyl 5HT (n=9) and 6-hydroxymelatonin (n=9) were below the limit of detection (200pcg) with the quantities of CSF used. The values of 5HIAA and tryptophan determined in the various disease groups are given below.

<u>DISEASE STATE</u>	<u>5HIAA</u> ng/ml	<u>TRYPTOPHAN</u>
Spinal Injury	15.71 (n=1)	137.60 (n=1)
ME	16.88 ± 1.59* (n=15)	329.62 ± 52.27* (n=15)
MS	16.79 ± 2.21* (n=6)	252.70 ± 52.93* (n=6)
BIH	16 (n=1)	323.60 (n=1)
MWD	17.24 ± 0.30* (n=2)	430.90 ± 225.70* (n=2)

* Values given as mean ± SEM.

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