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TECHNICAL NOTE

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The Presence of Trace Amines in Postmortem Cerebrospinal Fluid in Humans

ABSTRACT: The postmortem levels of biogenic amines in cerebrospinal fluid may represent a useful tool in defining some pathological conditions; no information is available concerning the occurrence of trace amines in postmortem cerebrospinal fluid. Thus, the occurrence of octopamine, synephrine and tyramine were evaluated by using a HPLC system in 20 postmortem samples of cerebrospinal fluid (obtained from 11 males and 9 females) and their levels were compared with those of 20 living subjects (obtained from 11 males and 9 females). The results show that trace amines dramatically increase in the postmortem cerebrospinal fluid (100, 20, and 4 fold increase for tyramine, octopamine, and synephrine respectively). To our knowledge, our data represent the first time trace amines have been identified in postmortem cerebrospinal fluid and the dramatic increase observed for tyramine has the potential of becoming a new tool in forensic science for better defining the time of death.

KEYWORDS: forensic science, trace amines, time of death, cerebrospinal fluid, high performance liquid chromatography, electrochemical detection

Tyramine, octopamine and synephrine are endogenous amines widely diffuse in plants, bacteria and invertebrates where they constitute the principal neurotrasmitters of the nervous system (1). In mammals, including man (2), they are ingested in substances that include chocolate, aged cheese and wine but are also endogenously synthesized by an alternative metabolic pathway of classic amines (dopamine, norepinephrine and serotonin). Nevertheless, these amines are present in mammals and humans in low quantities as compared to classic amines and, for this reason, they are called "trace amines" (3). Trace amines are stored in vescicles in the brain and in terminals of the autonomic system together with norepinephrine, dopamine (4) and serotonin (5-HT) and, once released, they may interfere, together with the classical biogenic amines, with the function of their receptors (5) since they behave like "false neurotrasmitters" (1,6).

For many years, the apparent lack of receptors that specifically bind trace amines has left these substances in a "shadow area" of human research. The recent discovery of a family of G protein–coupled receptors which bind and are activated by trace amines (called trace amine receptors, TARs) in vertebrates has opened up the possibility that they may constitute a new group of true neurotrasmitters (6). TARs have been shown to have a high affinity for trace amines, including tyramine and octopamine, and to differ from the classical biogenic amine receptors. These findings, in addition to the evidence that trace amines are present in various tissues and organs including specific brain areas such as the amygdala, hypothalamus and locus coeruleus (7), have suggested the possibility that one or more trace amines may also behave as a neurotransmitter or a neuromodulator in humans (8). Consistent with this hypothesis, it has been postulated that trace amines may play a role in different pathological conditions such as depression (9), hepatic encephalopathy (10), schizophrenia (11), hypertension (12) and in the pathogenesis of migraines (13).

In the forensic field, cerebrospinal fluid (CSF) concentrations of the monoamines and their metabolites are commonly used to provide information about central nervous system dopaminergic and serotonergic activity. In addition, both the experimental and clinical levels of catecholamines from tissue or CSF collected at different times after death are used to study catecholamine levels and their relationship to a given diagnosis, condition, treatment or situation (14–16). On the contrary, no information is currently available concerning the presence of trace amines in postmortem tissue or CSF.

The aim of the present study is to verify the presence of trace amines, namely tyramine, octopamine and synephrine, in the postmortem CSF of dead subjects. The levels of the catecholamines noradrenaline, dopamine and serotonin in the same postmortem CSF were also evaluated. The levels of CSF trace amines as well as those of CSF biogenic amines were compared with those found in the CSF of living subjects.

Materials and Methods

Subjects

CSF was taken from two groups of subjects. The first group consisted of 20 subjects (11 males and 9 females; mean age of 53.3 ± 8.7 years) selected from 50 cadavers who died from a

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cardiovascular pathology, thromboembolism, broncopneumonia or politrauma. These cases were collected between 2003 and 2004.

The second group (11 males and 9 females; mean age of 63.5 ± 5.5 years) was composed of 20 living subjects selected from 90 subjects who were hospitalized between January 2003 and December 2003. Their fluid was taken to answer clinical questions and none of these had problems of concentration or mental confusion, or pathological conditions such as a lymphoproliferative process, a neoplasm, or an inflammatory or degenerative disease.

The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Sampling of Cerebrospinal Fluid and Sample Preparation

CSF samples were taken by lumbar puncture performed by ventral perforation of intervertebral disk L4/L5. The CSF samples of the first group were taken in a postmortem interval that ranged from 24 to 72 h.

The CSF samples (2–3 mL) were taken using a 21 Gauge sterile needle with a 10 cc syringe and were then transferred to centrifugation tubes; after centrifugation at 2000 × g (10 min) to discard the cellular elements, the supernatants were stored at −20°C until utilized.

HPLC

The chromatographic system utilized for the determination of biogenic and trace amine levels has been previously described (8). Three incremental doses of 0.125, 0.250, 0.500 μg/mL 0.1 N perchloric acid of tyramine, octopamine and synephrine standard solutions (Sigma) were utilized to obtain the calibration curves. Standard solutions and extracts from biological tissues were injected for the identification and quantification of the catecholamines and trace amines. The retention time of tyramine, octopamine and synephrine were 18.50, 6.70 and 7.80 min (ratio accuracy 0.75, 0.88 and 0.87), respectively. The detection limits of the HPLC method were 22, 22 and 49 picograms for tyramine, octopamine and synephrine, respectively.

Statistical Analysis

Statistical analysis was performed using InStat software (Instant Biostatistics, Version 3.0 for Windows, GraphPadTM Software, Inc. 1990–98). Given the abnormal distribution (Kolmogorov and Smirnov normality test) of values, ordinary ANOVA using nonparametric methods was performed for all the comparisons. Values are expressed as the mean ± SEM.

Results and Discussion

Trace and biogenic amines were simultaneously detected in CSF samples. In the living subjects, the level of synephrine was comparable to that of noradrenaline (10.3 ± 4 and 11.9 ± 4 ng/mL of CSF, respectively). The trace amines octopamine and tyramine were also present in the CSF of the living subjects but their levels were much lower than synephrine. All the biogenic amines were found in the CSF of living subjects, the most concentrated being noradrenaline. The levels of the so-called trace amines and biogenic amines were not so different as expected; this likely reflects the high sensibility of the CoulArray HPLC employed. These data suggest that the use of the term "trace amine" is not appropriate and should be changed in the future. The high sensitivity of the method employed may also explain the higher level of catecholamine levels found in the present study as compared to previous studies (17–19). In the postmortem CSF, the levels of both the trace and the biogenic amines were elevated as compared to the CSF of the living subjects. The most dramatic increase involved tyramine whose levels were more than 100 fold higher. However, due to the wide range of concentrations, this increase was not statistically significant. The wide range of concentrations found for tyramine, as well as for serotonin, was very likely due to the fact that all biogenic and trace amine were measured simultaneously. Both tyramine and serotonin were detected as the final amines (retention times 16 and 26 min) and their values were probably underestimated in living subjects. This problem did not exist in postmortem since all values were higher and better detectable. A marked increase was also observed in the postmortem octopamine level that was 20 fold higher than in the living samples, while synephrine showed only a 4-fold increase. Among the biogenic amines, dopamine showed the highest increase (24 fold increase) in the post-mortem CSF while noradrenaline and serotonin showed a moderate increase (2 and 8 fold) (Fig. 1).

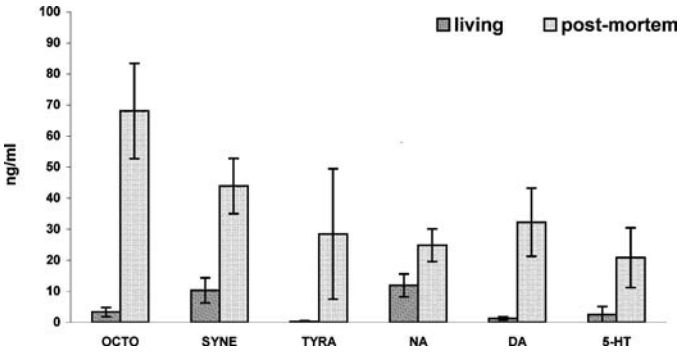

FIG. 1—*Levels of trace amines (octopamine, OCTO, synefrine, SYNE and tyramine, TYRA) and biogenic amines (noradrenaline, NA, dopamine, DA, and serotonin, 5-HT) in the CSF of living and dead subjects. Trace and biogenic amines were detected with HPLC as described in the material and methods section. Values are expressed as ng/mL CSF and represent the mean ± SEM of twenty independent determinations for each group. * = $p < 0.05$; ** = $p < 0.01$ Test.*

The results of the present study demonstrate the presence of the trace amines tyramine, octopamine and synephrine in human CSF and their dramatic increase following death. In addition, the data here reported confirm previous studies that have shown an increase of biogenic amines in postmortem CSF (15).

The finding concerning presence of trace amines and their increase in the postmortem CSF here reported raises fundamental issues concerning their production and their normal physiological role as well as their potential alterations in pathological conditions. The increasing levels of trace amines in postmortem CSF as compared to living subjects (as shown by Coul-Array HPLC technology) could be a sign of a possible metabolic shift at the expense of the catecholamines with which trace amines share the same substrate. Normal levels of these amines are, in fact, regulated by the catabolism of monoamino oxidases A and B which are decreased in depaupered energy cellular conditions such as human death. Monoamino oxidases are enzymes that normally metabolize biogenic amines and are correlated with the efficiency of the metabolic processes that lead to the final neurotransmitters such as gamma-aminobutyric acid (GABA), glutamate, dopamine and noradrenaline.

It is known that, in vivo, tyramine can be synthesized from tyrosine by the enzyme amino acid decarboxylase; tyramine can subsequently be transformed into octopamine, by dopamine-β-hydroxylase, and the octopamine into synephrine (the most stable

of the biogenic trace amines) by the enzyme phenylethanolamine *N*-methyltransferase. Synephrine is considered the final step of the trace amine metabolism (2). The deficiency of monoamino oxidase activity (20) could lead to a metabolic shift, activating inefficient pathways through aminoacidic decarboxylase instead of hydroxylase. The substances that could be found at the end of this metabolic process are the trace amines such as tyramine, octopamine, synephrine and others not investigated in the present study such as phenylethylamine, triptamine and their acidic metabolites (11).

In the CSF of living subjects, tyramine was the lowest trace amines and this finding is in line with the metabolic pathway of trace amines. The finding that it dramatically increased in postmortem subjects may reflect an up-regulation of aminoacid decarboxylase activity that is known to occur in the postmortem CSF (2).

The data reported may contribute to better understanding of the metabolic mechanism of death with the aim of identifying a metabolic pattern expression of this human condition and of attempting to identify the time of death by investigating the concentration of these amines at specific time intervals after the moment of death.

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