ONTOGENY OF *N,N*-DIMETHYLTRYPTAMINE AND RELATED INDOLEALKYLAMINE LEVELS IN NEONATAL RATS

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SUMMARY

The present study deals with the measurement of the brain levels of the two potent hallucinogens *N,N*-dimethyltryptamine (DMT) and 5-methoxy-*N,N*-dimethyltryptamine (OMB), the biogenic amine tryptamine (TA), and its condensation product 1,2,3,4-tetrahydro-beta-carboline (THBC) in rats of various ages.

Using gas chromatography–mass spectrometry with isotope dilution, we detected DMT, OMB, and THBC in neonatal rats from birth. DMT levels remained low until days 12 and 17 at which time they increased significantly and then returned to the initial low levels for all subsequent ages. The levels of OMB were higher than those measured for DMT with the highest levels being observed at days 12 and 17, and also on day 31. However, the levels for OMB showed much more variation. Although elevated levels of DMT and OMB have been correlated with stress, there are no known functions for these compounds. TA levels remained below detection limits until day 19. THBC levels were observed to be highest on days 22 and 31. The role that THBC plays in mammalian tissues is not known.

Key words: Ontogeny; *N,N*-dimethyltryptamine; *O*-Methylbufotenine; Tryptamine; 1,2,3,4-Tetrahydro-beta-carboline

INTRODUCTION

The hallucinogen *N,N*-dimethyltryptamine (DMT) has been identified as a normal constituent in human urine [1,2], blood [3,4], and cerebrospinal fluid [5,6]. Subsequently, DMT has been identified in adult rat adrenal and brain tissue [7]; however, the exact function or role that DMT plays in mammalian tissue has not yet been elucidated.

It has been reported that brain levels of DMT and its *O*-methyl cogener, 5-methoxy-*N,N*-dimethyltryptamine (OMB) in rats can be significantly elevated by various environmental stresses [7]. It has been shown that brain levels of tryptamine (TA) and its

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Pictet-Spengler condensation product, 1,2,3,4-tetrahydro-beta-carboline (THBC) are also elevated by stress [7]. The changes in the levels of these compounds with environmental conditions serve as an indication, at least, that there exist distinct mechanisms for the synthesis and release of these compounds in response to a given stimuli. Thus far studies examining endogenous levels of DMT, OMB, the biogenic amine TA and its condensation product THBC have been concerned with young-adult (60-day-old) or adult (120-day-old) rats. As has been shown, the levels of these compounds found in these rat brains vary depending upon the level of stress to which the animal is exposed and the housing conditions. In an unstressed, group-housed 60-day-old rat the levels of all of these compounds is very low (less than 2 ng/g wet tissue weight) or undetectable [7]. The occurrence of these compounds has not been reported in younger rat brain.

In attempts to describe the appearance and behavior of DMT, OMB, TA, and THBC, we examined brain levels of these compounds in developing rat brain using isotopic dilution gas chromatographic–mass spectrometric methods.

MATERIALS AND METHODS

Tissue procurement

Mixed-sex rat pups were generated from Sprague-Dawley rats (Charles River) bred in our animal facilities and the time of birth was noted (within a 3-h time range) for each litter. The animals were group-housed in a sound-attenuated room with a 12-h light/dark cycle until sacrifice. Groups of rat pups were sacrificed by decapitation at 1, 3, 6, 9, 12, 14, 17, 19, 22, 25, 27, 29, 31, 35, and 40 days after birth. The animals were sacrificed between 9:00 and 10:00 a.m. for all groups. After decapitation, individual tissues were pooled from 4–6 animals (for the younger animals) such that approximately 2.0 g of brain tissue was used for each sample. After pooling the tissue, the sample was frozen at −76°C until assayed for the indolealkylamines. Five samples were analyzed for each age tested.

Chemicals and reagents

Tryptamine (TA) was obtained form Aldrich Chemical Company and α,α,β,β-tetradeuterotryptamine ([$^2H_4$] TA)hydrochloride (98% isotopic purity) was obtained from Merck, Sharpe and Dohme. *N,N*-Dimethyltryptamine (DMT), 5-methoxydimethyltryptamine (OMB), α,α,β,β-tetradeutero-*N,N*-dimethyltryptamine ([$^2H_4$]DMT), and α,α,β,β-tetradeutero-5-methoxydimethyltryptamine [$^2H_4$]OMB) were gifts from Professors F. Benington and R. Morin of this laboratory. Tetrahydro-beta-carboline (THBC), and 1,2,3,4-tetradeutero-beta-carboline (T^{2}HBC) were gifts of Dr. S.A. Barker. Hepatafluorobutyrylimidazole (HFBI) was obtained from Pierce Chemical Co. and all other reagents were purchased from commercial sources and were of the highest available purity.

Sample extraction

Individual tared brain samples were homogenized (Polytron) in 2.5 ml of 7% perchloric

acid containing 1000 ng each of the deuterated internal standards for each compound analyzed. The volume was adjusted to 5.0 ml by the addition of glass distilled water and vigorously vortexed to ensure thorough mixing. The samples were centrifuged at 1000 *g* for 30 min to remove precipitated protein. The supernatant was decanted; the pellet was resuspended by the addition of 3.0 ml of 7% perchloric acid and rehomogenized. This was followed by a second centrifugation, and the supernatants were combined. The samples were then extracted and derivatized according to the methods previously described by this laboratory [5–8]. Linearity of recovery using this method of [$^2H_4$] DMT/DMT, [$^2H_4$] OMB/OMB, [$^2H_4$] TA/TA and [$^2H_4$] TDBC/THBC from rat brain has been shown to be linear over a wide range of concentrations [7]. The detection limit for all of these compounds is 1 ng [7,8].

Quantification

Mass spectra of derivatized DMT, [$^2H_4$] DMT, OMB, [$^2H_4$] OMB, TA, [$^2H_4$] TA, THBC, and T^{2}HBC were conducted using a Hewlett Packard 5985A gas chromatograph–mass spectrometer equipped with a data analysis system.

The mass spectra and selected ions for the identification and quantification of these compounds have been reported by this laboratory [5,8]. Pure standards of each compound were analyzed separately in the selected ion monitoring mode to determine the percentage interference between the ions. Quantification was accomplished by comparing ion ratios, correcting for interference, and then multiplying this value by the amount of internal standard added. This value was divided by the brain weight and expressed as ng of a specific indolealkylamine per g of wet tissue weight.

RESULTS

The mean values ± the standard deviation for DMT, OMB, TA, and THBC are given in Table I and are expressed in ng indolealkylamine per g wet brain tissue weight. Varying levels of all compounds were identified at most of the ages examined. DMT levels remained relatively low until day 12 at which time the levels significantly increased, peaking on day 17 and then returning to the lower levels seen initially. The levels of OMB measured were higher than those seen for DMT, but they were similar to those of DMT in that the level increased on day 12 and day 17. However, the level of OMB in brain reached its highest level on day 31 before falling off. Also, there were much higher standard deviations found with the OMB data. These are much larger than the standard deviations seen for DMT or THBC, and may reflect a much higher individual variability in OMB brain levels. We repeated a group of animals for day 17, but again found great variability in the OMB data.

TA could only be detected reliably on day 19. The levels found on the other days were at or below the detection levels for our method. Increased levels of THBC were observed on days 22 and 31.

DISCUSSION

Indolealkylamine levels have been shown to be increased by stress and environmental changes [7,9]. The adrenal glands appear to contain DMT, and the levels of DMT increase under stress ([7]; J.M. Beaton and P.E. Morris, unpublished data). It has also been shown that catecholamine levels are increased by stress. Kvetnansky *et al.* [10] have shown that adrenal epinephrine levels rose progressively throughout their study of neonatal rats and that noradrenaline levels showed a rapid increase in the adrenal glands of rats around 17 days old. It was shown that the activity of adrenal catecholamine-synthesizing enzymes also began to increase around day 17 [10]. In the present study, we observed high levels of DMT and OMB on days 12 and 17, which closely parallels the above findings. We were unable to measure the levels of DMT in the rat pup adrenal glands or serum, but have recently shown that the increases in brain DMT levels seen after stress are paralleled in the adrenal gland of the adult rat (Beaton and Morris, unpublished data). Elevated levels of these compounds may be the result of stress since this is the approximate time for the rat pups to open their eyes and to begin exploring their environment. Detectable levels of TA were observed on day 19. It may be possible that, prior to this time, any available TA was converted to THBC resulting in TA levels below the limit of detection. THBC levels peaked on days 22 and 31. It is around this time that the rat pups were weaned from the mother rats. This may be quite stressful to the pups. THBC levels

TABLE I

LEVELS OF DMT, OMB, TA, AND THBC AT VARIOUS AGES

Data are expressed as mean ± S.D. (ng/g wet weight).

Age (days)	*DMT*	*OMB*	*TA*	*THBC*
1	1.90 ± 0.88	11.48 ± 9.67	0.0	8.40 ± 2.89
3	2.13 ± 1.46	4.86 ± 3.75	–[b]	–[b]
6	3.83 ± 1.95	14.47 ± 2.57	–[a]	5.45 ± 2.33
9	2.28 ± 1.82	4.87 ± 5.45	–[b]	7.43 ± 2.72
12	8.58 ± 2.92	19.64 ± 4.43	–[b]	6.02 ± 2.45
14	5.20 ± 2.28	14.17 ± 1.79	–[b]	9.05 ± 3.00
17	17.50 ± 4.18	19.04 ± 13.07	0.0	1.17 ± 1.08
19	2.53 ± 1.59	7.36 ± 2.70	3.13 ± 1.77	6.78 ± 2.60
22	2.65 ± 1.62	9.82 ± 3.13	–[b]	18.81 ± 4.33
25	5.49 ± 2.34	4.62 ± 2.15	–[b]	0.0
27	1.40 ± 1.18	1.16 ± 1.07	0.0	2.00 ± 1.41
29	2.90 ± 1.70	0.0	0.0	6.54 ± 2.55
31	2.00 ± 1.40	32.2 ± 5.67	0.0	26.54 ± 5.15
35	1.25 ± 1.11	0.0	0.0	0.0
40	–[b]	5.20 ± 2.28	0.0	–[b]

[a] Peak interference with this sample.
[b] Below reliable detection limits.

have been shown to be elevated during stress [7]. However, as yet, there is no established function for THBC.

We have observed that two potent hallucinogens, DMT and OMB, and THBC are present at birth in rat pups. We have further shown that under stress such as eye-opening and weaning DMT, OMB, TA, and THBC levels are elevated and, after habituation to the stress, the levels of these compounds return to baseline levels.

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