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Neurobiochemical Alterations Induced by the Artificial Sweetener Aspartame (NutraSweet)[1,2]

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Neurobiochemical Alterations Induced by the Artificial Sweetener Aspartame (NutraSweet). COULOMBE, R. A., AND SHARMA, R. P. (1986). *Toxicol. Appl. Pharmacol.* 83, 79–85. The dipeptide aspartame (NutraSweet) is a newly approved and widely used artificial sweetener in foods and beverages. Consumption of aspartame (ASM) has been reported to be responsible for neurologic and behavioral disturbances in sensitive individuals. Unfasted male CD-1 mice were dosed orally with 13, 130, or 650 mg/kg ASM in corn oil, while control animals received corn oil alone. Three hours after dosing, the animals were killed, and the concentrations of the catecholamines norepinephrine (NE) and dopamine (DA), catecholamine metabolites 3-methoxy-4-hydroxymandelic acid (VMA), homovanillic acid (HVA), and dihydroxyphenylacetic acid (DOPAC), the indoleamine serotonin (5-HT), and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined by electrochemical high-performance liquid chromatography in six brain regions. ASM exerted its primary effect on adrenergic neurotransmitters in various brain regions. In the hypothalamus, the region richest in NE, increases in NE concentrations of 12, 49, and 47% were found in the low, medium, and high dose groups, respectively, relative to control. Significant increases of NE in the medulla oblongata and corpus striatum were also observed. Increases of the catecholamine DA and catecholamine metabolites VMA, HVA, and DOPAC were seen in various regions. The indoleamine serotonin and its metabolite 5-HIAA were unaffected by ASM treatment. These findings are consistent with ASM-induced increases in the brain catecholamine precursor amino acids phenylalanine and tyrosine, as reported earlier. Such observed alterations in brain neurotransmitter concentrations may be responsible for the reported clinical and behavioral effects associated with ASM ingestion.

Aspartame (L-aspartyl-L-phenylalanine methyl ester; NutraSweet) is a dipeptide recently approved by the U.S. Food and Drug Administration for use as an artificial sweetener. Aspartame (ASM) was discovered in 1965 and found to be 150–200 times as sweet as sucrose on a weight basis (Mazur, 1984). ASM is used in a variety of food products, but most importantly in diet carbonated beverages. Coincident with increased consumption, sales of ASM reached ca. $600 million in 1983, and are likely to increase dramatically in the future. Shortly following approval for use in carbonated beverages, reports of ASM-related neurological disturbances such as headaches, dizziness, mood alterations, alterations in menstrual patterns, gastrointestinal symptoms, and allergic-type reactions began to appear (Mass. Med. Soc., 1984).

Recent reports have indicated that oral administration of ASM is associated with large increases in whole-brain concentrations of phenylalanine (Phe) and tyrosine (Tyr) in the rat (Fernstrom *et al.*, 1983; Wurtman, 1983). Furthermore, it has also been demonstrated that when dietary carbohydrate is administered with ASM, the rise in brain Phe is roughly doubled compared to the effect of

[1] NutraSweet is a registered trademark of G. D. Searle & Co.

[2] Portions of this study have been presented previously (*The Pharmacologist* 27, 931, 1985).

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ASM alone (Wurtman, 1983). Since Phe and Tyr are precursors in the biosynthesis of adrenergic neurotransmitters such as norepinephrine (NE) and dopamine (DA), much of the research into possible health effects of ASM has focused on related neurobiochemical parameters. It has been postulated that consumption of ASM may potentially perturb the amounts of biogenic amines in the brain via ASM-induced increases in Phe and Tyr (Wurtman, 1983). However, Fernstrom *et al.* (1983) reported that there were no apparent alterations in whole-brain concentrations of NE, DA, or serotonin (5-HT) 60 min following a single oral dose of 200 mg/kg ASM in rats, although significant increases in serum and brain Phe and Tyr were seen.

Previous studies of possible ASM-induced neurotransmitter perturbations have involved determinations of neurotransmitter concentrations in whole-brain homogenates, which may not detect significant alterations in small but critical brain regions, such as the hypothalamus. The purpose of the present study was to assess the effect of oral ASM administration on neurotransmitter concentrations in discrete brain regions following microdissection of brain tissue.

METHODS

Chemicals. Aspartame, purchased from Sigma Chemical Company (St. Louis, Mo.), was mixed with corn oil, then homogenized gently to create a suspension. Corn oil was used as a vehicle to avoid solubility problems of ASM.

Animals and treatment. Male CD-1 mice (Simonsen Laboratories, Gilroy, Calif.) weighing 29–34 g were acclimated to the animal care facility, then randomly placed in treatment groups prior to the beginning of the study. The mice were maintained on rat chow (Wayne Products, Chicago, Ill.) and water *ad libitum*, and housed four per cage in a room maintained at 22 ± 1°C and 50 ± 10% humidity with a 12-hr light cycle.

Animals (four/group) received either 13, 130, or 650 mg/kg ASM in a volume of 0.25 ml; control animals received the same volume of homogenized corn oil only. Animals were killed 3 hr after dosing by decapitation, and the following brain regions were immediately sampled at 0°C: hypothalamus, medulla oblongata, cerebellum, corpus striatum, cortex, and midbrain (Glowinski and Iversen, 1966). The midbrain included the hippocamus, thalamus, and subthalamus regions (Coulombe and Sharma, 1985). Tissues were placed immediately in tared vials containing several volumes, in relation to tissue weight, of ice-cold 0.05 M $HClO_4$ with 0.5% cysteine, then kept frozen at -80°C until analyzed. To minimize possible diurnal variations in regional brain neurotransmitter concentrations, all animals were sampled between 10 AM and 12 noon on the same day.

Neurotransmitter analyses. Brain tissues were extracted and analyzed for concentrations of catecholamines, indoleamines, and metabolites by methods previously described (Mayer and Shoup, 1983; Coulombe and Sharma, 1985). Perchloric acid (0.05 M $HClO_4$ with 0.5% cysteine) extracts of individual samples were analyzed by electrochemical high-performance liquid chromatography (HPLC). The HPLC system consisted of a Bioanalytical Systems (West Lafayette, Ind.) Model LC 150 electrochemical analyzer with a Model LC 3A amphoteric detector, a Model LC 22A temperature controller, and a Biophase ODS column. The mobile phase was prepared as described (Mayer and Shoup, 1983; Coulombe and Sharma, 1985) and allowed to equilibrate overnight before sample analysis. Samples were eluted at 30°C at a flow rate of 1.5 ml/min^{-1}. The recovery of internal standards by this technique was ca. 100%, and the correlation coefficient of the standard curves was routinely >0.98. The data were analyzed by one-way analysis of variance followed by the least significant difference test, if the F ratios were significant (Ott, 1977). A $p < 0.025$ was accepted as statistically significant.

RESULTS

The regional concentrations of catecholamines and indoleamines in different brain regions were within ranges expected for the male CD-1 mouse as demonstrated from previous experiments (Sharma *et al.*, 1985).

Aspartame ingestion induced significant increases in catecholamine neurotransmitters in several brain regions. The effects were often maximal at 130 mg/kg with no further increases apparent at the highest dose. Aspartame exerted its greatest effect on NE in various brain regions (Table 1). In the hypothalamus, the brain region with the greatest concentration of NE, significant ASM-induced increases of 49 and 47% were found relative to control in the medium and high dose groups, respectively. A 12% increase in hypothalamic NE was also found in the low dose group, although this finding was not signifi-

TABLE 1

EFFECT OF ASPARTAME ON REGIONAL CONCENTRATIONS OF THE BRAIN CATECHOLAMINES, NOREPINEPHRINE (NE) AND DOPAMINE (DA)

Brain amine	Aspartame dose[b] (mg/kg)	Concentration as μg/g wet weight[a]					
		Hypothalamus	Medulla oblongata	Cerebellum	Midbrain	Corpus striatum	Cortex
NE	Control	1.449 ± 0.122	0.479 ± 0.025	0.255 ± 0.009	0.460 ± 0.042	0.268 ± 0.019	0.278 ± 0.026
	13	1.626 ± 0.176	0.604 ± 0.024[c]	0.278 ± 0.023	0.610 ± 0.101	0.595 ± 0.118[c]	0.239 ± 0.044
	130	2.157 ± 0.119[c]	0.530 ± 0.045	0.303 ± 0.021	0.527 ± 0.023	0.421 ± 0.035	0.320 ± 0.022
	650	2.134 ± 0.092[c]	0.651 ± 0.029[c]	0.292 ± 0.025	0.479 ± 0.057	0.416 ± 0.025	0.335 ± 0.009
DA	Control	0.568 ± 0.027	0.061 ± 0.010	0.031 ± 0.008	0.353 ± 0.111	6.528 ± 0.713	0.912 ± 0.168
	13	0.585 ± 0.055	0.071 ± 0.019	0.020 ± 0.002	0.322 ± 0.064	5.614 ± 1.470	1.060 ± 0.356
	130	0.821 ± 0.050[c]	0.044 ± 0.005	0.027 ± 0.002	0.210 ± 0.054	7.663 ± 0.738	0.883 ± 0.108
	650	0.784 ± 0.052[c]	0.069 ± 0.003	0.022 ± 0.003	0.230 ± 0.046	6.942 ± 0.795	0.784 ± 0.066

[a] Values are $\bar{x}$ ± SEM of four animals each.
[b] Animals killed 3 hr following a single oral dose.
[c] Significantly different relative to controls, $p < 0.025$.

cant. Significant increases in NE concentrations were found in other brain regions such as the medulla oblongata and corpus striatum, but not in the cerebellum, midbrain, and cortex (Table 1). Likewise, there were significant increases in hypothalamic DA in the medium and high dose groups, but no effects on DA concentrations were found in other brain compartments (Table 1). The apparent decreases of DA in the medulla oblongata and cerebellum (Table 1) were not significant as calculated by analysis of variance.

Concomitant with the observed increases in hypothalamic concentrations of NE and DA (Table 1), concentrations of the major catecholamine metabolites were found to likewise increase, primarily in the high dose groups (Table 2). Concentrations of 3-methoxy-4-hydroxy mandelic acid (VMA) significantly increased in the hypothalamus (Table 2), the region in which significant increases in the parent catecholamine NE were observed (Table 1). Similarly, significant increases in the concentration of homovanillic acid (HVA) in the medium dose group were seen in the hypothalamus (Table 2), presumably as a consequence of the increased parent catecholamine DA in that region. Furthermore, the concentration of dihydroxyphenylacetic acid (DOPAC) in the medulla oblongata was significantly increased in the high dose group.

Aspartame-related perturbations appeared to be confined to the catecholamine neurotransmitters, since the concentrations of the indoleamine serotonin (5-HT) and its metabolite 5-hydroxy-indoleacetic acid (5-HIAA) were unaffected (Table 3).

DISCUSSION

The doses employed in this study were chosen to closely approximate average intake as well as potential "overuse" amounts of ASM. The mean potential intake of ASM in the 2- to 4-year age group is 12 mg/kg (Federal Register, 1983). The acceptable daily intake (ADI) of ASM is 40 mg/kg for adults (Stegnik, 1984). Since it has become apparent that ASM consumption by a large segment of consumers is

TABLE 2

EFFECT OF ASPARTAME ON REGIONAL CONCENTRATIONS OF THE BRAIN CATECHOLAMINE METABOLITES 3-METHOXY-4-HYDROXY MANDELIC ACID (VMA), HOMOVANILLIC ACID (HVA), AND DIHYDROXYPHENYLACETIC ACID (DOPAC)

Brain amine	Aspartame dose[b] (mg/kg)	Concentrations as μg/g wet weight[a]					
		Hypothalamus	Medulla oblongata	Cerebellum	Midbrain	Corpus striatum	Cortex
VMA	Control	0.395 ± 0.017	0.381 ± 0.017	0.388 ± 0.020	0.431 ± 0.033	0.484 ± 0.051	0.402 ± 0.033
	13	0.449 ± 0.022	0.418 ± 0.028	0.458 ± 0.035	0.523 ± 0.056	0.694 ± 0.186	0.333 ± 0.083
	130	0.412 ± 0.045	0.402 ± 0.010	0.391 ± 0.011	0.526 ± 0.055	0.505 ± 0.050	0.403 ± 0.017
	650	0.534 ± 0.060[c]	0.438 ± 0.028	0.412 ± 0.006	0.393 ± 0.082	0.491 ± 0.021	0.376 ± 0.014
HVA	Control	0.144 ± 0.012	0.051 ± 0.002	0.034 ± 0.004	0.205 ± 0.058	0.901 ± 0.081	0.190 ± 0.031
	13	0.147 ± 0.026	0.060 ± 0.006	0.034 ± 0.002	0.169 ± 0.020	0.830 ± 0.119	0.188 ± 0.036
	130	0.234 ± 0.024[c]	0.048 ± 0.008	0.034 ± 0.004	0.143 ± 0.030	1.108 ± 0.104	0.184 ± 0.018
	650	0.214 ± 0.005	0.058 ± 0.006	0.029 ± 0.002	0.125 ± 0.040	0.914 ± 0.077	0.176 ± 0.019
DOPAC	Control	0.166 ± 0.031	0.074 ± 0.008	0.047 ± 0.004	0.181 ± 0.031	0.957 ± 0.098	0.191 ± 0.027
	13	0.127 ± 0.015	0.071 ± 0.002	0.049 ± 0.003	0.179 ± 0.026	0.756 ± 0.129	0.177 ± 0.032
	130	0.231 ± 0.036	0.065 ± 0.003	0.051 ± 0.002	0.147 ± 0.023	1.169 ± 0.092	0.182 ± 0.017
	650	0.206 ± 0.013	0.093 ± 0.003[c]	0.053 ± 0.003	0.155 ± 0.021	0.921 ± 0.110	0.190 ± 0.011

[a] Values are $\bar{x} \pm$ SE of four animals each.
[b] Animals killed 3 hr after a single oral dose.
[c] Significantly different relative to controls, $p < 0.025$.

TABLE 3

EFFECT OF ASPARTAME ON REGIONAL CONCENTRATIONS OF SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA)

Brain amine	Aspartame dose[b] (mg/kg)	Concentrations as μg/g wet weight[a]					
		Hypothalamus	Medulla oblongata	Cerebellum	Midbrain	Corpus striatum	Cortex
5-HT	Control	1.044 ± 0.089	0.674 ± 0.021	0.275 ± 0.015	0.874 ± 0.032	0.567 ± 0.107	0.591 ± 0.034
	13	0.994 ± 0.136	0.776 ± 0.023	0.254 ± 0.009	1.155 ± 0.214	0.969 ± 0.160	0.482 ± 0.095
	130	1.322 ± 0.112	0.655 ± 0.039	0.265 ± 0.013	0.774 ± 0.097	0.665 ± 0.089	0.572 ± 0.026
	650	1.214 ± 0.027	0.736 ± 0.029	0.239 ± 0.018	0.826 ± 0.012	0.643 ± 0.047	0.559 ± 0.009
5-HIAA	Control	0.643 ± 0.061	0.419 ± 0.030	0.173 ± 0.008	0.664 ± 0.048	0.624 ± 0.144	0.267 ± 0.032
	13	0.519 ± 0.090	0.434 ± 0.033	0.145 ± 0.031	0.726 ± 0.108	0.484 ± 0.065	0.207 ± 0.021
	130	0.873 ± 0.117	0.374 ± 0.032	0.155 ± 0.012	0.552 ± 0.091	0.537 ± 0.051	0.243 ± 0.018
	650	0.663 ± 0.011	0.412 ± 0.009	0.141 ± 0.013	0.570 ± 0.035	0.432 ± 0.017	0.231 ± 0.055

[a] Values are $\bar{x}$ ± SE of four animals each.
[b] Animals killed 3 hr following a single oral dose.

probably higher than these values (Wurtman, 1983), the doses used in this study are a reasonable representation of these conditions. In the current study, corn oil was used as a vehicle, and it is conceivable that ASM in an aqueous vehicle may have produced greater biological effects due to a more rapid absorption.

The observed neurotransmitter increases, exclusively in the regional concentrations of NE, DA, and their metabolites, suggest a specific ASM-induced perturbation in the adrenergic biosynthetic pathway. The changes in NE concentrations in the hypothalamus were highly significant at the 133 and 650 mg/kg doses of ASM, in spite of the limited number of animals in each group. Similar trends have been observed in other experiments in our laboratory with a similar protocol. Furthermore, increases in the adrenergic metabolites HVA, VMA, and DOPAC (Table 2) strengthen the validity of these results. Because concentrations of NE and DA are controlled by the amounts of the precursor amino acids Phe and/or Tyr (for review, see Wurtman *et al.*, 1980), it is likely that the observed increases of NE and DA, as well as of the catecholamine metabolites VMA, HVA, and DOPAC, resulted from an ASM-caused increase in brain Phe, a component and an important degradation product of ASM.

This hypothesis is supported by recent studies demonstrating that oral administration of ASM (200 mg/kg) in rats induced large increases in whole-brain Phe and Tyr (Wurtman, 1983; Fernstrom *et al.*, 1983). Since Phe is rapidly converted to Tyr—at least half of the Phe in protein is converted to Tyr in each passage through the hepatic circulation (Elwyn, 1970)—subsequent conversion of Tyr to catecholamines in the brain may proceed shortly after ASM ingestion. Furthermore, it is also known that dietary supplementation of Phe at relatively low concentrations (1%) is responsible for significant increases in locomotor activity in rats, presumably via concomitant increases in brain NE and DA (Thurmond *et al.*, 1977).

It should be emphasized that the observed ASM-induced increased concentrations of catecholamine neurotransmitters and their metabolites should be corroborated with the direct measurement of their turnover rates.

Often, ASM-induced increases in various neurotransmitters seemed to peak at the medium dose with no further increases apparent at the highest dose. A possible explanation for this trend may be end-product inhibition of tyrosine hydroxylase. However, it has never been shown that brain catecholamine concentrations can increase sufficiently to inhibit tyrosine hydroxylase, except when animals are treated with a monoamine oxidase inhibitor (Wurtman *et al.*, 1980).

The precise health implications of increased brain NE and DA following ASM ingestion remains unclear. However, of the many neurotransmitters known to exist in the central nervous system, the catecholamines NE and DA have most often been linked to the behavioral pathology of a number of disorders (Antelman and Caggiula, 1977). Alterations in brain NE and DA have been related to the onset in convulsive seizures (Kohsaka *et al.*, 1978). Pharmacologically active agents such as tetrahydrocannabinol and methamphetamine are often associated with increases in brain NE and DA (Poddar *et al.*, 1976; Koda and Gibb, 1973). Furthermore, basic activities such as eating (Antelman and Caggiula, 1977; Barnett, 1980), reproductive behavior (Gessa and Tagliamonte, 1974), stress-related aggression (Geyer and Segal, 1974), and locomotor function (Thurmond *et al.*, 1977) appear to be mediated by brain concentrations of NE and/or DA. In addition, the general modulating effect of NE and DA on the central nervous system has been suggested to influence mood and the feeling of well-being (Reinis and Goldman, 1982).

Many of the above roles of central NE and DA in behavior appear to arise due to the involvement of these catecholamines in the hypothalamic secretion of hypophysiotrophic hormones. For example, hypothalamic concentrations of NE and DA, either directly or indirectly, have important modulating effects on vasopressin, oxytocin, prolactin, luteinizing hormone (LH), growth hormone, and thyroid-stimulating hormone (TSH) (Reichlin *et al.*, 1978; Hutchinson, 1978; Krulich, 1979). Thus many of the aforementioned symptoms reportedly related to ASM consumption, i.e., headaches, dizziness, mood alterations, and menstrual pattern alterations, may be attributable to increases in regional brain catecholamine concentrations.

To our knowledge, the effects of ASM on neurotransmitters in various brain regions have not been investigated. Fernstrom *et al.* (1983) showed that although ASM (200 mg/kg) caused large increases in blood and brain Phe and Tyr in the rat, no significant changes in brain NE, DA, 5-HT, DOPAC, HVA, and 5-HIAA were detected. However, since the analyses were performed in whole-brain homogenates, significant alterations in minute brain compartments, e.g., the hypothalamus, may have been masked and, therefore, undetected. It is evident that neurotransmitter determinations in microdissected brain tissue are essential when one is interested in screening for potential perturbations of these chemicals.

Concentrations of regional brain neurotransmitters are important in the modulation of behavior, particularly in light of the critical regulatory role of the hypothalamus, the brain regions where the observed effects of ASM were most profound. It is therefore possible that ASM consumption may produce neurobiochemical and behavioral effects in humans, particularly in children and susceptible individuals. Based on the foregoing, there is a need for additional research on the safety of this food additive.

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