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SHORT COMMUNICATIONS

Lack of effect of chronic aspartame ingestion on aminergic receptors in rat brain

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The sweetener aspartame (APM, L-aspartyl-L-phenylalanine methyl ester) has been studied in detail to establish its safety as a food additive. At the present time, there is no convincing evidence to suggest adverse effects of this compound for the general population [1–4].

In the gastrointestinal tract, APM is metabolized rapidly to aspartic acid and phenylalanine (Phe); the Phe is partially biotransformed in the liver to tyrosine (Tyr). Large amounts of APM must be ingested to moderately elevate plasma levels of these amino acids in humans [5] or in laboratory animals [6–8]. However, by altering the "plasma ratio" of Phe or Tyr to other amino acids within the same transport class, the entry of other large neutral amino acids such as tryptophan (Trp) into the CNS can be affected [8]. Since Phe, Tyr, and Trp are precursors of the neurotransmitters norepinephrine (NE), dopamine (DA), and 5-hydroxytryptamine (serotonin, 5-HT), changes in brain content of these amino acids may alter neurotransmitter concentrations [9, 10] and subsequently affect receptor binding in the CNS.

This paper reports studies on the influence of chronic administration of APM on binding kinetics of CNS dopaminergic, adrenergic, and serotonergic receptors. In contrast to earlier studies, which examined effects of acute APM administration, the present study investigated longer term effects by making APM available to rats in the drinking water. This ingestion more closely resembles human consumption of very large amounts [i.e. up to ten times the acceptable daily intake (ADI)] of APM.

Materials and methods

Materials. [^{3}H]Prazosin (sp. act. 26 Ci/mmol), [^{3}H]clonidine (46.4 Ci/mmol), [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT, 157 Ci/mmol), [^{3}H]ketanserin (61 Ci/mmol), [^{3}H]SCH-23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine HCl, 70 Ci/mmol), and [^{3}H]spiperone (21.4 Ci/mmol) were from Dupont NEN Research Products (Boston, MA). Butaclamol, SCH-23390, and 8-OH-DPAT were from Research Biochemicals Inc. (Natick, MA); clonidine, 5-HT, and polyethylenimine were from the Sigma Chemical Co. (St Louis, MO); methysergide was from Sandoz Pharmaceuticals (East Hanover, NJ). Phentolamine was provided by Ciba/Geigy (Summit, NJ). Aspartame, provided by the NutraSweet Co. (Deerfield, IL), was 98% pure APM (other components included: 0.08% α-aspartyl-phenylalanine, 0.15% aspartyl phenylalanine diketopiperazine, and 0.25% β-aspartyl-phenylalanine methyl ester). All other chemicals were of reagent grade.

Aspartame administration. Male Sprague–Dawley rats (purchased at 140–160 g body weight from Charles River, Williamstown, MA) were housed two per cage under standard conditions with a 12-hr light/dark cycle (6:00 a.m. to 6:00 p.m. lights on) and unlimited access to laboratory chow (Formulab Chow 5008, Ralston Purina Co., Richmond, IN) and water or APM (50 or 500 mg/kg per day for 30 days) in water. Body weight and water consumption were monitored daily, and drinking solutions were prepared to provide the intended dose of APM in the expected volume consumed. Neither the gain in rat body weight nor the amount of water consumed during the 30-day treatment period was affected by APM administration (data not shown). Fresh APM solutions were prepared every 3–4 days. APM solutions were adjusted to pH 4.0 to prevent degradation; however, spontaneous degradation occurs very slowly at room temperature [2]. High-performance liquid chromatographic studies demonstrated no breakdown of APM in the drinking water during the experimental period (data not shown). Tap water for control rats was also adjusted to pH 4.0.

Receptor kinetics and statistical analysis. At the end of 30 days of ingestion of APM at doses of 50 and 500 mg/kg per day, rats were decapitated in the early morning (at 7:00 to 8:00 a.m.) and brains were rapidly removed, chilled, and dissected into cerebral cortex, corpus striatum, and hippocampus. Fresh tissue combined from two rats was assayed immediately for receptor kinetics. Published procedures were used to study binding of [^{3}H]prazosin to adrenergic α_1 receptors [11], of [^{3}H]clonidine to α_2 receptors [12], of [^{3}H]8-OH-DPAT to 5-HT$_{1A}$ receptors [13], and of [^{3}H]ketanserin to 5-HT$_2$ receptors [14]. Dopamine D$_1$ and D$_2$ receptors were examined using [^{3}H]SCH-23390 [15] and [^{3}H]spiperone [16] respectively. Protein content in tissue homogenates was determined by the method of Lowry *et al.* [17].

The affinity (K_d) and the maximum number of binding sites (B_{max}) were determined by a nonlinear least-squares curve-fitting algorithm of the LIGAND computer program [18] as modified for use on IBM hardware by G. A. McPherson and distributed by Elsevier-Biosoft. Potential differences between treatment conditions were evaluated by one-way analysis of variance (ANOVA). The treatment means were compared via the Newman–Keuls test.

Results

Table 1 illustrates that the binding kinetics at the six amine neurotransmitter receptors studied were unaltered by chronic ingestion of APM. In cerebral cortical membrane preparations, the kinetic parameters of [^{3}H]prazosin (α_1) and [^{3}H]clonidine (α_2) binding were not affected by APM at doses of 50 and 500 mg/kg per day, nor was the binding of [^{3}H]ketanserin to the 5-HT$_2$ receptors. The binding of [^{3}H]SCH-23390 to D$_1$ receptors and [^{3}H]spiperone to D$_2$ receptors, in the striatum, was also not altered by APM consumption. Since the receptors tested showed no change at an APM dose of 50 mg/kg, the binding of [^{3}H]8-OH-DPAT to 5-HT$_{1A}$ receptors of the hippocampus was studied only in the brain of rats given 500 mg/kg of APM per day. Neither K_d nor B_{max} values were altered.

Discussion

Concern has been expressed that APM ingestion may induce adverse neurological responses via alterations in CNS neurotransmitters [19]. It has been suggested that changes in Phe, Tyr, and Trp content could affect neurotransmitter function and thus induce behavioral abnormalities. Although many published studies (see Ref. 20 for review) have found no untoward effects attributable to APM consumption, the few reports of neurochemical effects [8, 21, 22] prompted this study of amine neurotransmitter receptors after extended APM exposure. A

Table 1. Effect of chronic APM administration on dopaminergic, adrenergic, and serotonergic receptor binding kinetics

[^{3}H]Ligand (Receptor)	Control		APM (50 mg/kg per day)		APM (500 mg/kg per day)	
	K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)
SCH-23390 (D_1)	0.55 ± 0.10	780 ± 54	0.52 ± 0.10	774 ± 48	0.60 ± 0.10	806 ± 62
Spiperone (D_2)	0.06 ± 0.01	279 ± 15	0.07 ± 0.01	284 ± 11	0.05 ± 0.01	250 ± 17
Prazosin (α_1)	0.05 ± 0.01	203 ± 11	0.07 ± 0.02	207 ± 21	0.04 ± 0.01	215 ± 25
Clonidine (α_2)	2.5 ± 0.2	115 ± 8	2.3 ± 0.3	101 ± 8	2.1 ± 0.3	100 ± 11
8-OH-DPAT (5-HT_{1A})	2.2 ± 0.3	167 ± 14			2.6 ± 0.6	163 ± 20
Ketanserin (5-HT_2)	0.54 ± 0.04	269 ± 34	0.62 ± 0.13	272 ± 46	0.60 ± 0.10	284 ± 54

Male Sprague–Dawley rats had unlimited access to aspartame (APM) in their drinking water for 30 days at concentrations yielding doses of 50 and 500 mg/kg per day. All animals were killed between 7:00 and 8:00 a.m. [^{3}H]Prazosin, [^{3}H]clonidine, and [^{3}H]ketanserin binding studies were carried out in cerebral cortical tissue. Tissue from the hippocampus was used in studies of [^{3}H]8-OH-DPAT binding. All dopaminergic binding ([^{3}H]SCH-23390 and [^{3}H]spiperone) kinetic parameters were evaluated in striatal tissue. Values for each kinetic parameter are the means ± SE of 6–12 individual determinations. No K_d or B_{max} value (APM treatment vs control), as determined by ANOVA, was statistically significant ($P > 0.1$).

major difference between previous studies and this one is the method of APM administration: other investigators gave single large doses, whereas we allowed *ad lib.* ingestion in the drinking water, which was followed by excision of the brain early in the day, i.e. at the end of the rats' period of greatest activity and water consumption (data not shown). The absence of any changes in receptor binding kinetics after 30 days of administration of 50 or 500 mg/kg APM to rats suggests that APM does not alter the function of these systems. We have also demonstrated that this chronic consumption of APM does not alter hippocampal or cerebral cortical concentrations of NE, 5-HT, and DA or striatal concentrations of DA [23]. We are not aware of any other studies of the effect of prolonged APM administration on receptor kinetics.

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Nathan S. Kline Institute for Psychiatric Research
Orangeburg, NY 10962
U.S.A.

MARGARET A. REILLY*
EDMUND A. DEBLER
ARTHUR FLEISCHER
ABEL LAJTHA

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* Corresponding author.

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Uroporphyria caused by acetone and 5-aminolevulinic acid in iron-loaded mice

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A number of polyhalogenated aromatic compounds, such as hexachlorobenzene (HCBZ*), cause hepatic uroporphyria in experimental animals that is accompanied by decreased activity of hepatic uroporphyrinogen decarboxylase (URO-D) [for review see Refs 1 and 2]. A similar human condition occurred in Turkey in the late 1950s when HCBZ-treated wheat seed was ingested [1]. More recently, it has been found that uroporphyria can be produced in iron-loaded mice by polycyclic aromatic hydrocarbons that do not contain halogen atoms: Francis and Smith reported that continuous feeding of 3-methylcholanthrene (MC) and other polycyclic aromatic hydrocarbons for 10 weeks produces uroporphyria in iron-loaded mice [3], whereas Urquhart *et al.* reported that a single injection of MC causes uroporphyria in iron-loaded mice given 5-aminolevulinic acid (ALA) in their drinking water for 9 days [4]. Both the halogenated compounds and polycyclic aromatic compounds are inducers of cytochrome P450s of the IA subfamily. Cytochrome P450IA2, which is inducible in the rat by MC and HCBZ, catalyzes the oxidation of uroporphyrinogen, an activity that we have suggested is involved in the development of the uroporphyria [5]. In contrast, phenobarbital, an inducer of the IIB cytochrome P450, was inactive [4] or weakly active [3] in causing porphyria in the iron-loaded mice. In this paper, we report that acetone caused a massive uroporphyria and decreased hepatic URO-D activity in iron-loaded mice given 5-aminolevulinic acid in their drinking water. Although acetone is a highly effective inducer of cytochrome P450 of the IIE and IIB subfamilies in the rat [6], we found no evidence that the uroporphyria in acetone-treated mice was due to uroporphyrinogen oxidation catalyzed by these forms of cytochrome P450.

Materials and methods

Animals. Male C57BL/6 mice (22–25 g) were purchased from Charles River Breeding Laboratories (Wilmington, MA), and were fed RMH 3000 rodent chow (Purina) *ad lib.* Iron (12.5 mg/mouse) was administered i.p. as Imferon (iron-dextran, Carter-Golgau, Phoenix, AZ). Both acetone [ACS, Fisher (Fairlawn, NJ); 1% (v/v)] and ALA [Sigma Chemical Co., St Louis, MO; 2 mg/ml] were given continuously in the drinking water. (The details of the experiments are described in the table legends.) All mice drank approximately 2 ml liquid/day. MC (Sigma) was given i.p. in approximately 0.3 ml corn oil at 130 mg/kg, 48 hr before killing the animals, and sodium phenobarbital was given i.p. in approximately 0.3 ml saline at 100 mg/kg, once each day for 3 days before killing. Animals were kept in temperature-controlled rooms with 12-hr dark/light cycles. The animals were killed by cervical dislocation, and the livers were removed and homogenized in 4 vol. of 0.25 M sucrose. The homogenates were centrifuged at 10,000 *g* for 10 min, and then a portion of the supernatant fraction was centrifuged at 100,000 *g* for 1 hr for preparation of microsomes. Microsomes were washed with 0.15 M KCl, and the pellet was stored at −60° for up to 4 weeks before assay. These storage conditions did not affect enzyme activities.

Enzyme assays. Uroporphyrinogen decarboxylase activity was assayed in 10,000 *g* supernatant fractions as described [7] using 5 mM pentacarboxyporphyrinogen III as substrate. Aniline hydroxylase was assayed as described [8] using 0.15 mM aniline as substrate. The plot of the rate of the reaction was biphasic as a function of substrate concentration as has been found for hamster [9] and chicken hepatocytes [8]. Uroporphyrinogen oxidation was measured as described [5]. Cytochrome P450 was measured by the method of Omura and Sato [10]. Microsomal ethoxy- and pentoxyresorufin dealkylase activities were assayed as described [11]. Porphyrins were determined spectrofluorometrically [12] or by HPLC as described [13].

Results and discussion

Table 1 shows that high levels of URO accumulated in the livers of mice when treated with the combination of iron-dextran, acetone and ALA. Livers from three of the five mice had URO concentrations greater than 290 nmol/g wet wt. The urinary excretion of URO in animals given all three compounds was also elevated, being detectable

* Abbreviations: ALA, 5-aminolevulinic acid; HCBZ, hexachlorobenzene; MC, 3-methylcholanthrene; URO, uroporphyrin; and URO-D, uroporphyrinogen decarboxylase.