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Aspartame and seizures

Review Article

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Summary. It has been hypothesized that the dietary sweetener aspartame (L-aspartyl-L-phenylalanine methyl ester) might promote seizures and this hypothesis has been argued in the published literature. The current manuscript reviews the biochemical, neurochemical and behavioral experiments that have been carried out in order to assess the hypothesis linking aspartame with seizure promotion. We conclude that convulsive seizures are not caused by orally administered aspartame in rodents or in primates, including humans. Early reports of seizure facilitation by aspartame in several rodent models were not confirmed by later and more careful experimentation. Proconvulsive effects were absent in humans and other mammals with epilepsy and those without epilepsy. Lack of convulsive liability was evident, even when doses many fold higher than those consumed in the human diet, were used in experimental paradigms. Studies of aspartame in absence seizures are not as complete as those in convulsive seizures, but available evidence in humans does not document an association between absence seizure incidence and aspartame usage.

Keywords: Amino acids – Seizures – Genetic epilepsy – Aspartame – Phenylalanine – Neurotransmitters

1 Introduction

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is used as a dietary sweetener throughout much of the world (Butchko and Kotsonis, 1989; Bickel and Trefz, 1986). Metabolic investigations in humans and other animals show that the substance is hydrolyzed in the gastrointestinal tract to L-phenylalanine, L-aspartic acid and methanol rather than being absorbed as an intact molecule (Ranney et al., 1976).

The possibility that aspartame promotes seizures has been argued in published literature (Chiu and Woodbury, 1988; Fountain et al., 1988; Kim et al., 1988; Pinto and Maher, 1988; Walton, 1986; Wurtman, 1985). Many of these

proconvulsant arguments have been predicated upon presumed reductions in brain noradrenergic and/or serotonergic transmission that might be caused by aspartame ingestion. Prior to the speculations concerning aspartame and seizures, a large body of data had developed showing that the level of noradrenergic and/or serotonergic activity in the central nervous system modulates the propensity to seizure activity. High monoaminergic activity is anticonvulsant, whereas low monoaminergic activity is proconvulsant. Accordingly, aspartame would have a convulsant liability if it produced a sufficient reduction in monoaminergic activity.

The present manuscript has been prepared as an examination of the pertinent data and issues pertaining to aspartame and seizures. Because initial speculations about the seizure promoting effects of aspartame were based on proposed neurotransmitter alterations produced by conversion of the sweetener to phenylalanine, aspartate and methanol, pertinent neurochemical mechanisms of seizure facilitation will also be examined.

2 Neurotransmitters and seizure induction

Many neurotransmitter systems have been proposed to play a role in seizures. Importance in the etiology of seizure disorders has frequently been imputed to the noradrenergic, dopaminergic, serotonergic and glutamatergic systems (Faingold and Naritoku, 1991; Fisher, 1991; Fisher and Coyle, 1991; Jobe et al., 1991a,b). They have the potential to modulate seizure thresholds, seizure severity and other aspects of seizure activity in epileptic and non-epileptic animals. Moreover, neuronal systems using excitatory amino acids as transmitters have the capacity to initiate seizures in specific regions of the brain.

2.1 Noradrenergic systems

Most studies in non-epileptic animals have demonstrated that a reciprocal relationship exists between experimentally-induced changes in noradrenergic transmission and seizure activity (Maynert et al., 1975; Browning, 1987a; Jobe and Laird, 1987). Similarly, innate presynaptic and postsynaptic noradrenergic deficits contribute to the epileptic condition of the genetically epileptic animals. Evidence supporting a noradrenergic deficit hypothesis of epilepsy in the genetically epilepsy-prone rats (GEPRs) is especially persuasive (Dailey et al., 1989b; Jimenez-Rivera and Waterhouse, 1991; Jobe et al., 1991a,b, 1992b; Laird, 1989). Data also support a similar noradrenergic hypothesis of seizure predisposition in the epileptic baboon and some evidence supports the hypothesis that humans with epilepsy may be characterized similarly by noradrenergic deficits (see Jobe et al., 1991b for a recent review). Descriptions of these genetically epileptic rodents and primates are presented subsequently in Section 6.1 of the current monograph.

Experimentally-induced noradrenergic deficits facilitate both forebrain and brainstem seizures in non-epileptic rats. Commonly, tonic extensor convulsions induced by corneal electroshock are used as an index of brainstem seizures (Browning, 1987b). The threshold for these seizures is unequivocally reduced by treatments which diminish noradrenergic transmission. Moreover, the extension/flexion ratio associated with maximal electroshock is augmented by

noradrenergic deficits. A treatment-induced increase in the extension/flexion ratio is an index of brainstem seizure facilitation (Browning, 1987b; Swinyard, 1972).

The facilitation of forebrain seizures by noradrenergic deficits in non-epileptic rats has also been demonstrated. Neurotoxic lesions of noradrenergic pathways lower the threshold for facial and forelimb clonus, an index of forebrain seizure activity (Mishra et al., 1991). Moreover, desipramine, a norepinephrine reuptake inhibitor, increases extracellular norepinephrine (Mishra et al., 1989; Yan et al., 1992a) and elevates the threshold for facial and forelimb clonus (Mishra and Jobe, unpublished data). Both pathophysiologic and pharmacologic data support the concept that innate noradrenergic deficits in the GEPR contribute to the seizure prone condition both of forebrain and brainstem seizure circuitry (Browning et al., 1989; Mishra et al., 1991; Jobe et al., 1991c; Wang et al., 1990).

In addition to the foregoing evidence pertaining to convulsive seizures, experimentation also documents a similar relationship between facilitation of absence seizures and decrements in noradrenergic activity. Accordingly, drug-induced decrements in noradrenergic transmission cause increases in spike-wave discharges in the genetically absence epilepsy rat [GAER] (Micheletti et al., 1987). An exception to this role for noradrenergic fibers has been identified in the tottering mouse by Levitt and Noebels (1981). These animals are notable, partially because they were discovered as the first genetic rodent model of spontaneous non-convulsive seizures, that is of absence-like seizures with spike-wave EEG discharges (Green and Sidman, 1962; Noebels and Sidman, 1979). Also, they exhibit convulsions characterized by ataxia and intermittent focal myoclonus. Rather than innate deficits in noradrenergic indices, these animals have a selective axonal overgrowth in noradrenergic neurons that appears to contribute to the epileptic condition (Levitt and Noebels, 1981).

In contrast to the seizure facilitating effects of drug-induced noradrenergic decrements, experimentally induced noradrenergic increments are anticonvulsant both in non-epileptic animals and in genetically epileptic subjects (Jobe et al., 1991a,b). Also, recent transplantation paradigms suggest that appropriately localized injections of locus ceruleus cells in GEPRs protect the animals against seizures (Clough et al., 1991). Evidence indicates that the injected noradrenergic cells successfully innervate the innately deficient terminal fields of the GEPR brain, thereby partially restoring normal noradrenergic activity.

2.2 Serotonergic systems

Serotonergic influences within the brain also regulate seizure propensity (Jobe et al., 1991a,b; Segal, 1991). For example, in the GEPR, drug-induced decrements in serotonergic activity increase audiogenic seizure severity. In contrast, experimentally-induced serotonergic increments are anticonvulsant. In this regard, the amino acid serotonin precursor 5-hydroxytryptophan produces a dose related anticonvulsant effect against audiogenic seizures when administered to GEPRs pretreated with a monoamine oxidase inhibitor (Jobe et al., 1973a). Similarly, fluoxetine (a drug with high specificity for blocking the reuptake of serotonin into nerve terminals) is an effective anticonvulsant in GEPRs (Dailey

et al., 1992b). Evidence obtained with the use of intracerebral microdialysis technology shows that the magnitude of the anticonvulsant effect of fluoxetine is closely linked to its induction of increases in serotonin concentrations in the extracellular fluid. Another recent study with intracerebral microdialysis indicates that carbamazepine, a drug used extensively in the treatment of generalized tonic/clonic seizures and partial seizures in humans, may exert at least part of its anticonvulsant effect by augmenting serotonin release in the brain (Yan et al., 1992b).

Innately, the GEPR is characterized by serotonergic deficits which appear to act as partial determinants of seizure predilection (Dailey et al., 1992a; Jobe et al., 1982, 1991a,b; Statnick et al., 1991). Similarly, serotonergic deficits may be of etiologic significance in some epileptic mice, the epileptic baboon and in some human seizure disorders (Jobe et al., 1991b; Giroud et al., 1990).

2.3 Glutamatergic systems

Glutamate and aspartate appear to function as excitatory neurotransmitters at numerous synapses in the brain (Fisher, 1991; McGeer and McGeer, 1989; Meldrum, 1991). Indeed, glutamate is believed to be the primary fast excitatory neurotransmitter in the central nervous system (Meldrum, 1991). Glutamatergic pathways participate in the generation and propagation of epileptic seizures (Delgado-Escueta et al., 1986; Faingold and Naritoku, 1991; Wasterlain, 1988). Five types of excitatory amino acid receptors have been identified in mammalian brain: NMDA receptors; quisqualate receptors; kainate receptors; AP4 receptors; and ACPD receptors (Browning, 1991; McGeer and McGeer, 1989). Glutamate and aspartate are mixed agonists at each of these receptors.

Excessive amounts of glutamate and/or aspartate in the brain cause seizures, excitotoxicity and neuronal death (Browning, 1991). Direct intracerebral application of glutamate, aspartate or specific excitatory amino acid receptor agonists will produce seizures in mammals (Smialowski, 1983; Faingold and Meldrum, 1990; Faingold and Naritoku, 1991).

3 Methanol and seizures

Methanol is a byproduct of the intestinal hydrolysis of aspartame which occurs in man and other animals (Ranney et al., 1976). As such, individuals who consume aspartame sweetened products can be expected to absorb some intact methanol. Methanol, like a number of other alcohols, is widely distributed in nature. It is a normal constituent of a number of eatable fruits (Lund et al., 1981; Kazeniac and Hall, 1970; Kirchner and Miller, 1957; Heatherbell et al., 1971) and is found in human saliva and expired air (Eriksen and Kulkarni, 1963; Larsson, 1965). Because early investigators found considerable variability in the concentration of methanol in expired air, it was hypothesized that dietary sources account for only part of the total body pool of methanol (Eriksen and Kulkarni, 1963). This hypothesis was confirmed when it was found that methanol is formed in mammalian tissues (Axelrod and Daly, 1965; Kim, 1973; Morin and Lis, 1973; Gagnon, 1979; Gagnon and Heisler, 1979).

The amounts of methanol available to be absorbed after aspartame ingestion are very small and are most likely biologically insignificant (Stegink, 1984; Fisher, 1989; Leon et al., 1989). For example, the amount of methanol available from an aspartame sweetened beverage (obtained from commercial sources for dietary use) is less than the methanol found in an equal volume of fruit juice (Stegink, 1984). That is, if an aspartame-sweetened beverage contained a typical concentration of 555 mg of aspartame per liter, there would be 60 mg of methanol available to be released on hydrolysis. This 60 mg/liter of methanol available from the aspartame in a beverage is less than the average of 140 mg/liter of methanol found in fruit juices (Stegink, 1984; Lund et al., 1981; Kazeniac and Hall, 1970; Kirchner and Miller, 1957; Heatherbell et al., 1971).

The central nervous system depression produced by methanol is qualitatively similar to that seen after ethanol. Quantitatively, however, the inebriating effects of methanol are less pronounced than those of ethanol (Tephly and McMartin, 1984). Methanol overdoses are known to produce serious toxicities including intense abdominal pain, blindness, respiratory depression, coma, convulsions and death. However, these toxicities result from the metabolic conversion of methanol to formate and not from the parent compound (McMartin et al., 1977). Convulsions following methanol intoxication occur when the patient is near death, if they occur at all.

Recently, Leon et al. (1989) carried out a double-blind placebo controlled trial in which they administered 75 mg/kg/day of aspartame to volunteers for 24 weeks. This dose is 1.5 times the 50 mg/kg/day of aspartame considered acceptable under current FDA guidelines (Federal Register 49: 6672-6682, 1984) and is approximately thirty times the 90th percentile average daily consumption (Butchko and Kotsonis, 1991). Even with these very large doses, Leon and colleagues (1989) could not detect a difference in trough blood methanol or formate concentration between aspartame treated and placebo treated subjects. Additionally, there was no difference in 24 hour urinary formate concentration between controls and aspartame treated subjects. In view of these data, it seems as if the toxic liability from methanol formed from dietary aspartame is nonexistent.

4 Phenylalanine, tyrosine and seizures

Phenylalanine is an essential dietary ingredient in that it cannot be synthesized by mammals and is an indispensable nutrient. It is converted to tyrosine by phenylalanine hydroxylase, an enzyme concentrated in liver, but also found in kidney and pancreas. This irreversible conversion to tyrosine represents the major step in the degradation of excess phenylalanine (Harper, 1984). Phenylalanine and tyrosine are necessary for protein synthesis while tyrosine is the precursor for the catecholamine neurotransmitters and neurohormones. Dopamine, norepinephrine and epinephrine are neurotransmitters in brain. Norepinephrine is the neurotransmitter in the peripheral sympathetic nervous system and norepinephrine and epinephrine are released from the adrenal medulla.

Phenylalanine and tyrosine enter the brain via an amino acid transport system. There are specific transport systems, with limited overlap, for acidic,

small neutral, large neutral and basic amino acids (Neame, 1968; Blasberg and Lajtha, 1965; Harper, 1984). Phenylalanine, tyrosine, leucine, isoleucine, valine, tryptophan, histidine and methionine all are transported mainly by the large neutral amino acid transport system. Because they share the same transport system, the large neutral amino acids (LNAAs) compete with each other for entry into the brain. A measure of this competition is provided by the ratio of one of the amino acids to the sum of the remaining LNAAs (Fernstrom, 1983).

It has been hypothesized that phenylalanine, derived from dietary aspartame, might facilitate seizures. The basis of this hypothesis is that increased plasma phenylalanine would compete for transport and decrease the brain entry of tyrosine and tryptophan (Wurtman, 1985; Pinto and Maher, 1988). According to this hypothesis, decreasing brain tyrosine and tryptophan would result in a decrease in synthesis of neurotransmitters for which tyrosine and tryptophan are obligatory precursors. Norepinephrine, epinephrine and dopamine are brain neurotransmitters synthesized from tyrosine while serotonin is synthesized from tryptophan. However, any effect that the phenylalanine from aspartame may have on the ratio of phenylalanine to the other LNAA's (Phe/LNAA) is not unique to aspartame. Ingestion of carbohydrate or protein can also affect Phe/LNAA (Fernstrom et al., 1979). For example, ingestion of sucrose leads to a release of insulin and, consequently, to decreases in the plasma concentrations of valine, leucine and isoleucine. Such insulin effects on the concentrations of these LNAA's lead to changes in this ratio of similar magnitude as occur after equal sweetness amounts of aspartame (Martin-Du Pan et al., 1982; Burns et al., 1991; Wolf-Novak et al., 1990).

A corollary also has been added to this hypothesis: Once an increased concentration of phenylalanine has entered the brain, it will decrease the synthesis of the catecholamines. This corollary was derived from indirect observations. First, tyrosine and not phenylalanine is the primary substrate for tyrosine hydroxylase, the rate limiting enzyme in the synthesis of the catecholamine neurotransmitters. Second, phenylalanine is a weak competitive inhibitor of tyrosine hydroxylase *in vitro*. From these two bits of information, it was surmised that an increase in the quantity of phenylalanine relative to tyrosine may result in competition for tyrosine hydroxylase so as to reduce the synthesis of epinephrine, norepinephrine and dopamine. Hypothetically, a decrease in serotonin synthesis would occur solely because competition at the blood brain barrier decreases availability of tryptophan in the brain. Such presumed reductions in brain catecholamines and/or serotonin have been hypothesized to be at least partially responsible for the postulated effects of aspartame on seizure thresholds in individuals consuming large quantities of the sweetener (Pinto and Maher, 1988; Wurtman, 1985).

One test of the above hypothesis would be to administer a wide range of oral doses of aspartame to epileptic subjects whose seizure predisposition is dependent, in part, on abnormalities in monoaminergic function. If the hypothesis was correct, brain tyrosine and tryptophan entry would be decreased and brain catecholamine and serotonin concentrations would be reduced. Also, the reductions in neurotransmitter levels would cause the epileptic subjects to experience an increase in seizure incidence and/or severity.

After large oral doses of aspartame in rats, there are dose-dependent increases in plasma phenylalanine and tyrosine. These increases lead to increases in concentration of both phenylalanine and tyrosine in the brain (Yokogoshi et al., 1984; Fernstrom et al., 1983; Pinto and Maher, 1988; Dailey et al., 1991). A decrease in brain concentration of tyrosine was not detected upon administration of acute doses of aspartame as high as 2000 mg/kg. In all of the studies in which it was reported, brain tyrosine concentration increased after large doses of aspartame.

For humans, the acceptable daily intake (ADI) established by the Food and Drug Administration is 50 mg/kg (Federal Register 49: 6672-6682, 1984). This dose would be equivalent to consumption of 18 to 20 cans of aspartame-sweetened beverage by a 70 kg adult (Sze, 1989; Fisher, 1989). Actual average aspartame consumption, however, is only 2 to 3 mg/kg/day, even at the 90th percentile (Butchko and Kotsonis, 1991). Thus, the aspartame doses used in animal studies [as high as 3000 mg/kg in rats (Jobe et al., 1992a), 2500 mg/kg in mice (Dailey et al., 1989a) and 1000 mg/kg in baboons (Meldrum et al., 1989)] are as much as 1000 times current consumption levels.

Some authors have emphasized that higher doses of aspartame are justified in rodents because these animals have liver phenylalanine hydroxylase activity which is 5 fold higher than do humans (Yokogoshi et al., 1984). Maher and Wurtman (1987) have suggested that, in rodents, the greater amount of tyrosine formed from phenylalanine serves as an "antidote" to the effects one might expect from phenylalanine. They have proposed that in order to mimic the human situation, doses of aspartame should be 60 fold higher in rodents (Wurtman and Maher, 1987). On this basis, it was asserted that doses of aspartame up to 3000 mg/kg (60 x the ADI of 50 mg/kg) might be appropriate. However, Fernstrom has reviewed this issue and questioned the appropriateness of such assertions (Fernstrom, 1989). Recently, the relationship between aspartame dose and plasma phenylalanine concentration was examined in mice and rats over a wide range of doses (Hjelle et al., 1992). The results of this study indicate that the pharmacokinetics of phenylalanine and tyrosine and the ratio Phe/LNAA are dose related. A comparison of these ratios with those from humans indicates that rodents require approximately 2 to 6 times more aspartame to attain similar plasma Phe/LNAA. Thus, although studies in animals were done with doses of aspartame up to 3000 mg/kg, doses of aspartame as low as 4 to 18 mg/kg in rodents would be equivalent to current human exposure (Butchko and Kotsonis, 1991).

To account for the higher phenylalanine hydroxylase activity in rodents as compared to humans, the original hypothesis concerning aspartame and seizures could be restated for rodents as follows:

Very large doses of aspartame (2000 mg/kg or more as an oral bolus) might be expected to increase plasma phenylalanine and tyrosine such that the transport ratios of phenylalanine, tyrosine and tryptophan and the ratio of phenylalanine to tyrosine concentration in blood are comparable to the corresponding ratios in humans consuming extremely large amounts of aspartame in the diet (perhaps up to 100 or 150 mg/kg/day in divided doses). Further, when the appropriate

ratios are achieved, entry of phenylalanine into the brain will be increased, whereas entry of tyrosine and tryptophan will be decreased. Ultimately, decreased levels of tyrosine and tryptophan will reduce the synthesis, storage and release of serotonin and catecholamine neurotransmitters to produce changes in seizures in rodents.

Recent findings which address the "large dose hypothesis" in rodents are scrutinized subsequently in Section 6.0. In general, "large dose" papers which have included brain monoamine assays as part of their experimental protocols have shown that concentrations of norepinephrine and dopamine do not decrease in response to doses of aspartame between 1000 and 3000 mg/kg orally (Dailey et al., 1989a; 1991; Jobe et al., 1992a). Moreover, unpublished observations from experiments with intracerebral microdialysis in our laboratory indicate that release of norepinephrine and dopamine from neurons does not change in response to 2000 mg/kg in rats. Neither do the extracellular concentrations of catecholamine metabolites (DOPAC and HVA) and the serotonin metabolite (5-HIAA). In addition, investigators from other laboratories have reported similar findings. From *in vivo* voltammetry experiments in rats, Peregro et al. (1988) have demonstrated that 1000 mg/kg aspartame does not alter indices of brain neurotransmission. Garattini and coworkers (1988) demonstrated by microdialysis that 1000 mg/kg of aspartame did not affect release of monoamine neurotransmitters.

Failure of large aspartame doses to reduce stores and release of norepinephrine occurs despite two other aspartame-induced events that had previously been hypothesized to produce noradrenergic decrements. *First*, phenylalanine transport ratios in rats rise to higher levels than those of tyrosine at doses of 2000 mg/kg or greater (e.g. at the 2500 mg/kg dose, the phenylalanine transport ratio is approximately 380% of the tyrosine transport ratio) (Dailey et al., 1991; Jobe et al., 1992a). *Second*, phenylalanine/tyrosine ratios rise from a fasting control level of approximately 0.98 to 2.9 in severe seizure GEPRs (GEPR-9s) treated with 3000 mg/kg of aspartame. Interestingly, this elevated phenylalanine/tyrosine ratio appears larger than that seen in humans treated with a dose of 34 mg/kg of aspartame given in a single dose rather than in divided doses throughout the day (as would be the case with dietary consumption). Accordingly, data reported by Stegink (1984) show that this human ratio is approximately 1.69 following a single bolus dose of 34 mg/kg. Moreover, at 50 mg/kg and 100 mg/kg, the ratio in humans is 2.0 and 2.2, respectively. Clearly, the ratio of 2.9 in GEPR-9s treated with 3000 mg/kg exceeds that seen in humans given enormous bolus doses of aspartame many times current consumption. Indeed, the doses above 1000 mg/kg in rodents are equivalent to abuse doses in humans. If aspartame daily doses of 34, 50 and 100 mg/kg were divided into fractions given at intervals throughout a 24 hour period, pharmacokinetic principles predict that the peak level of phenylalanine and tyrosine would be lower than after a single dose. These comparisons provide confirming evidence for the concept that the large dose aspartame paradigms in rodents are adequate for testing for seizure liability.

These large dose studies in rodents validated another prediction of the hypothesis. They produced decreases in tryptophan transport ratios in non-epileptic CD-1 mice, non-epileptic rats and in the moderate and severe seizure GEPRs (that is, GEPR-3s and GEPR-9s). The reduced transport ratios were associated with some small decrements in serotonin concentrations in some areas of the brain (Dailey et al., 1989a, 1991). But previous observations indicate that seizure enhancement requires much larger reductions in serotonin concentrations in response to synthesis inhibition (Dailey et al., 1989a; Jobe et al., 1973a).

In view of these neurochemical observations, it is not surprising to note that the large dose paradigms in rodents did not result in seizure enhancement in the studies cited above. The predicted noradrenergic decrements did not occur and the serotonergic decrements were too small to influence seizure responsiveness.

The significance of these observations in rodents can be interpreted further in light of the experiments conducted by Meldrum and coworkers where the effects of large doses of aspartame and phenylalanine were examined in the epileptic baboon (Meldrum et al., 1989). This primate epilepsy model is different from the rodent models and similar to humans in that it does not have high liver phenylalanine hydroxylase activity. In the experiments of Meldrum and colleagues (1989), doses of phenylalanine of 50 to 450 mg/kg or of aspartame of 300 or 1000 mg/kg were given orally. The highest doses of each agent increased plasma phenylalanine transport ratios by 30 fold, yet seizure alterations were not detected.

Subsequent sections of this manuscript will describe some experiments in which aspartame was reported to facilitate seizures. These findings will be evaluated from the context of experimental design and significance. In some of the studies, the gastrointestinal tract was bypassed by administering the aspartame moiety parenterally or directly onto brain tissue so that relevance to dietary use in humans was obviated (orally administered aspartame is degraded before entering the blood). A few instances of aspartame-induced seizure facilitation may be attributable to other elements of experimental design including the well known Type I error inherent in multiple univariate tests. As would be anticipated, these reported observations have not been replicated in subsequent experiments.

5 Excitatory amino acids and seizures

The possibility that the dietary consumption of aspartame might have proconvulsant liability has usually been attributed to factors other than the increases in aspartic acid in plasma and brain. Although excitatory amino acids unequivocally produce seizures (Arauz-Contreras and Feria-Velasco, 1984; Beas-Zarate et al., 1985, 1989; Bhagavan et al., 1971; Johnston, 1973; Mushahwar and Koeppe, 1971; Nemeroff and Crisley, 1975; Stewart et al., 1972), the doses required far exceed those that might be achieved by dietary consumption of aspartame as a sweetener. Single intraperitoneal doses of glutamate commonly associated with seizures are between 3000 and 4000 mg/kg (Arauz-Contreras and Velasco, 1984). Bradford and Dodd (1977) observed that glutamate doses below 3670 mg/kg rarely produced convulsions in adult rats, whereas doses of

3855 mg/kg almost always caused convulsions. Also, they reported that the doses of aspartic acid associated with convulsions were similar to those of glutamate. Forty percent (w/w) of the aspartame molecule is represented by the aspartic acid moiety. Thus, delivery of 3000 mg/kg of the excitatory amino acid would require an acute aspartame dose of 7500 mg/kg. This dose is approximately 3000 times the 90th percentile average daily aspartame consumption (Butchko and Kotsonis, 1991).

An estimate of the threshold concentration of blood glutamate required to produce convulsions in rats is provided by the observations of Bradford and Dodd (1977). These investigators found that, following the intraperitoneal administration of glutamate, the blood level of this excitatory amino acid at the time of first appearance of convulsions was 42000 nmol/ml. This value is 39622% of the pretreatment glutamate concentration determined by Bradford and Dodd (1977). In humans, concentrations of plasma glutamate achieved after bolus doses of aspartame up to 40 times the 90th percentile average daily aspartame consumption are not significantly above fasting levels (Stegink, 1984). At 1 hour post administration, oral doses of aspartame as high as 500 mg/kg do not significantly elevate plasma aspartic acid concentrations in the GEPR (Dailey et al., 1991). Aspartame doses of 2000 mg/kg result in aspartic acid levels of approximately 180 nmol/ml in severe seizure GEPRs and 95 nmol/ml in moderate seizure GEPRs. Even at this 2000 mg/kg dose of aspartame and despite 5 to 16 fold increases in plasma aspartic acid levels, the concentrations of this amino acid in the GEPR brain did not increase. In humans, 200 mg/kg of aspartame as a bolus resulted in small increases in plasma aspartic acid and glutamic acid concentrations. However these concentrations remained well within the normal postprandial range (Stegink, 1984). Thus, even when aspartame is ingested in single, acute doses that are approximately 80 to 800 times the 90th percentile daily intake for use as a sweetener, aspartic acid and glutamic acid concentrations in humans and other mammals remain far below the levels known to cause seizures.

Since excitatory amino acids cause neuronal damage in addition to seizures in rodents if ingested in sufficient doses, consideration of aspartame in this context is warranted. Accordingly, plasma concentrations of aspartic acid exceeding 1100 nmol/ml cause hypothalamic neuronal necrosis in neonatal rodents (Daabees et al., 1985; Olney, 1969; Lemkey-Johnston and Reynolds, 1974; Okaniwa et al., 1979). Such toxicities also occur when plasma concentrations of glutamate exceed 750 nmol/ml or the combined glutamic plus aspartic acid levels exceed 1280 nmol/ml. Olney and colleagues have also reported toxicity in neonatal non-human primates given large bolus doses of glutamate (Olney and Sharpe, 1969; Olney et al., 1972). But attempts to confirm these findings in other primate studies have been unsuccessful (Abraham et al., 1971, 1975; Reynolds et al., 1971, 1979, 1984; Newman et al., 1973; Wen et al., 1973; Stegink et al., 1975; Heywood and James, 1979). A comparison of the aspartic and glutamic acid levels associated with neuronal damage and those associated with aspartame ingestion in doses as high as 2000 mg/kg in rats and 200 mg/kg in humans shows that the peak excitatory amino acid concentrations are considerably below levels associated with neuronal necrosis in neonatal rodents.

6 Experimental observations pertaining directly to aspartame and seizures

The initial speculations imputing seizure liability to aspartame peaked the interest of a number of laboratories. The substance was already in general use as a dietary sweetener in several countries including the United States of America and others were considering formal approval for dietary use. Studies specifically designed to determine whether seizure liability is a property of aspartame have now been completed in mice, rats and baboons. Both epileptic and non-epileptic subjects within each species have been evaluated for responses to aspartame.

6.1 Studies in epileptic mammals

Genetically epileptic mammals including humans exhibit seizures spontaneously and/or in response to stimuli that do not cause seizures in normal subjects (Jobe et al., 1991b). Moreover, many epileptic individuals appear to have an exaggerated responsiveness to seizure inducing stimuli that also cause seizures in non-epileptic subjects. The epileptic mammals that have been used as models for study of aspartame and seizure liability are characterized by these same types of seizure predilection.

6.1.1 Epileptic mice

Several strains of mice are genetically epileptic (Jobe et al., 1991b). Their epileptic predisposition is expressed either as susceptibility to spontaneous or environmentally induced seizures. The DBA/2 strain is the most widely studied of the genetic mouse models of epilepsy (Chapman and Meldrum, 1987). Loud sound is an effective seizure producing stimulus in these animals. DBA/2 mice have abnormally low thresholds for maximal electroshock (Ludvig et al., 1985), and the convulsant drug flurothyl (Davis and King, 1966; Marley et al., 1986). Occasionally DBA/2 mice exhibit convulsions in response to handling (Chapman and Meldrum, 1987).

6.1.1.1 Bicuculline-induced seizures in DBA/2 mice

Chiu and Woodbury (1988) reported in an abstract that single acute aspartame doses altered the convulsive dose in 50% of the animals (CD_{50}) for bicuculline-induced tonic and also clonic seizures in DBA/2 mice. Because of space constraints inherent in abstracts, directions and magnitudes of changes were not entirely clear and the statistical significance of parts of the data were not set forth. Clarification was not feasible at the annual meeting of the American Society for Pharmacology and Experimental Therapeutics because the poster was not presented.

In our own laboratory we have tested aspartame for effects on bicuculline-induced seizures in 27 day-old DBA/2 mice (Bettendorf et al., 1989). The incidence of clonus with loss of posture was used as the seizure endpoint. Aspartame doses were: 0, 10, 30, 300 and 1000 mg/kg. Twenty-five DBA/2 mice were assigned to each dosage group. Four hours following the oral administration of

aspartame, the animals were injected intraperitoneally with a previously determined CD_{50} (3.49 mg/kg; clonus with loss of righting). The convulsive incidence was calculated for each group. Only the 10 mg/kg dose was associated with a significant effect: an increase in incidence of clonic seizure. In an attempt to replicate the findings, we limited our protocol to a comparison of a 10 mg/kg aspartame group with a vehicle group and increased the size of each of the two groups to 58 (total for the experiment was 116). The seizure incidence in the treatment group did not differ significantly from that of the vehicle group. We concluded that aspartame doses up to 1000 mg/kg do not cause facilitation of bicuculline-induced clonic convulsions in DBA/2 mice. Our decision was based on three factors: (1) the significant increase in incidence noted in the first experiment was limited to only one of 4 aspartame treated groups; (2) the significant difference was noted at the smallest dose of aspartame without any evidence of greater doses producing a greater effect. Indeed the effect was lost with all higher doses; and (3) our second experiment designed to replicate the small dose effect produced negative results despite using more than enough animals to double the sample size.

6.1.1.2 Audiogenic seizures in DBA/2 mice

DBA/2 mice are susceptible to sound-induced seizures for a discrete period during their growth and development (see Jobe et al., 1992b for a review). Two groups have evaluated the effects of aspartame in DBA/2 mice during the developmental period in which they are susceptible to audiogenic seizures. In an abstract, Chiu and Woodbury (1988) reported that aspartame-treated DBA/2 mice had a significantly higher audiogenic seizure intensity at 30 minutes and one hour after aspartame administration. They also reported that audiogenic seizure intensity was "lower" at 2, 4 and 8 hours after aspartame administration. Because of the cryptic style necessitated by the abstract format, the dose(s) of aspartame were not clearly stated. The audiogenic seizure data are presented by Chiu and Woodbury (1988) in the same abstract used for the bicuculline data (see Section 6.1.1.1) so that additional insights were not available. As noted above, the scheduled presentation by the authors did not occur at the Society meeting.

Our laboratory evaluated the effects of a range of aspartame doses on audiogenic seizure incidence and intensity in 27-day-old DBA/2 mice, and the results have been reported in abstract form (Jobe et al., 1989a,b). In one experiment, the first sound stimulus was given 5 minutes before vehicle or aspartame gavage (5, 10, 30, or 100 mg/kg) in order to test the hypothesis that seizures might increase entry of aspartame metabolites into the brain (Jobe et al., 1989b). In response to the first stimulus, 75% of the mice exhibited audiogenic seizures. No dose of aspartame was associated with increases in convulsion incidence or intensity. Indeed, none of the vehicle or aspartame-treated animals had a convulsion when they were sound stimulated 1, 2, 4 or 8 hours after aspartame gavage. Thus, multiple acoustic stimuli did not alter convulsion intensity or incidence in DBA/2 mice treated with aspartame.

In a second study, 27-day-old DBA/2 mice were given doses of aspartame up to 2000 mg/kg (Jobe et al., 1989a). In three separate experiments, audiogenic

seizures were evaluated at 30 minutes, two hours and four hours after aspartame or vehicle gavage. No dose of aspartame was associated with a significant alteration in convulsion incidence or intensity at any of the time points. These experiments support the concept that aspartame does not produce proconvulsant or anticonvulsant effects against audiogenic seizures in DBA/2 mice.

6.1.1.3 Electroshock seizures in DBA/2 mice

Again, in the same abstract cited in the preceding two sections, Chiu and Woodbury (1988) stipulate that an oral aspartame dose of 200 mg/kg elevates minimal electroshock seizure threshold in DBA/2 mice. However, they also point out that this anticonvulsant effect of aspartame was not dose dependent when doses of 10 to 1000 mg/kg were evaluated. Unpublished observations in our laboratory also suggest a marginal anticonvulsant effect of aspartame (10 to 2000 mg/kg) against minimal electroshock seizures. In our first protocol with minimal electroshock, the anticonvulsant effect appeared to be dose dependent. But in three attempts to replicate the finding, statistical significance was inconsistently present and the effect was not dose dependent. In total, our four experiments included 74 mice with vehicle treatment and 372 with aspartame treatment. We suspect that in DBA/2 mice the possible anticonvulsant effect of aspartame is slight and subject to masking by environmental variables common in the breeding and husbandry programs of suppliers and in the routine of the laboratory setting.

6.1.2 Epileptic rats

Genetically epilepsy-prone rats (GEPRs) have been utilized in several different paradigms designed to determine whether aspartame administration causes or facilitates seizures (Dailey et al., 1987, 1988, 1991; Jobe et al., 1992a). These animals were chosen for testing specifically because of their special suitability (Dailey et al., 1991): (1) the animals are genetically epileptic; (2) the underlying causes of their epilepsy include deficits in neurochemical systems which aspartame has been hypothesized to influence; and (3) previous studies have shown that drug-induced alterations in neurotransmission within these systems also cause consistent and readily detectable changes in seizure indices.

Point 1 – Genetic epilepsy: GEPRs are characterized by a marked degree of seizure predisposition, a trait that they share with human epileptics (Jobe et al., 1991b). Both experience spontaneous seizures, both exhibit an exaggerated responsiveness to convulsant stimuli such as electroshock or pentylenetetrazol, and both have seizures in response to stimuli which do not cause seizures in neurologically normal subjects.

Point 2 – Neurochemical determinants of epilepsy: The etiologically significant neuronal systems that have been hypothesized as being especially vulnerable to the influence of aspartame are the noradrenergic and serotonergic systems. Innate deficits in these two systems characterize the GEPR brain and the epileptic condition of GEPRs is partially dependent on deficient transmission in both systems. Aspartame has been hypothesized to be proconvulsant because

of speculations that it might diminish both noradrenergic and serotonergic transmission. Moreover, some evidence suggests that abnormalities in noradrenergic and serotonergic transmission may contribute to epileptic states in humans (Jobe et al., 1991b).

Point 3– Responsiveness of seizure-prone traits to drug-induced modification of etiologically significant neuronal systems: A large body of evidence documents the responsiveness of seizure end points in GEPRs to drugs which alter noradrenergic or serotonergic activity (Dailey et al., 1989b; Jobe et al., 1991a,b). For example, an increase in either noradrenergic or serotonergic transmission suppresses audiogenic seizure severity. In contrast, decrements in transmission exacerbate audiogenic seizure severity.

6.1.2.1 Audiogenic seizures

Dailey and colleagues (1991) examined the effects of aspartame on audiogenic seizures in two strains of GEPRs, one characterized by moderate seizures and the other by severe seizures (Jobe et al., 1991a,b). Animals from the moderate seizure colony (GEPR-3s) exhibit clonic convulsions in response to sound stimulation, whereas subjects from the severe seizure colony (GEPR-9s) exhibit tonic extensor convulsions when exposed to the acoustical stimulus.

The study of Dailey and coworkers (1991) evaluated the effects of aspartame on three aspects of audiogenic seizures: (1) the audiogenic seizure severity score (ARS); (2) the latency from onset of the sound stimulus to the onset of the prodromal running episode; and (3) the latency from onset of stimulation to the onset of clonic or tonic convulsions. With these assessments, seizure facilitation is detectable by an increase in the ARS and/or a decrease in the latency to running and/or to convulsion. The rationale for the structure of the ARS system, which was developed by Jobe et al. (1973b) for ranking the severity of audiogenic seizures, is elaborated in recent reviews (Jobe et al., 1991a,b).

Studies of the effects of aspartame on audiogenic seizures in GEPRs were coupled with assessments of the plasma and brain amino acid concentrations and brain monoaminergic neurotransmitters. Both acute and subchronic paradigms were used for aspartame administration. In the acute experiments, GEPR-3s and GEPR-9s were fasted for 17 hours before vehicle or aspartame were given by gavage. Doses of 10, 100, 200, 1000 and 2000 mg/kg were administered 1 hour before seizure assessments. In the subchronic experiments, groups of GEPR-3s and GEPR-9s were given free access to solutions of aspartame as their only drinking fluid. Consumption of the liquid was determined at daily intervals and GEPRs were exposed to the acoustical stimulus once every 7 days for 4 weeks in order to determine seizure indices. In this subchronic paradigm, the average daily doses of aspartame in GEPR-3s and GEPR-9s combined were approximately 40, 85, 250, 445 and 825 mg/kg. The highest mean daily dose consumed was in GEPR-9s, the measured value being 863 mg/kg.

Dose related increases in plasma aspartic acid, tyrosine and phenylalanine were produced by acute administration of bolus doses of aspartame by gavage both in GEPR-3s and GEPR-9s. Subchronic administration of aspartame was not associated with alterations in plasma amino acid concentrations in samples

taken between 08:00 and 16:00 hours (unpublished observations). However, acute aspartame administration caused substantial increases in the tyrosine and phenylalanine transport ratios. In contrast, significant decrements in the tryptophan transport ratios were produced in GEPR-9s but not in GEPR-3s.

Despite these alterations in amino acids, no changes occurred at any dose of aspartame in any of the convulsion indices either in GEPR-3s or GEPR-9s. This absence of effect on audiogenic seizures was apparent both in the acute and sub-chronic experiments.

6.1.2.2 Pentylenetetrazol-induced seizures

Dailey and colleagues (1988) administered vehicle or 2000 mg/kg of aspartame orally to GEPR-3s and GEPR-9s. One hour after this treatment, the tonic CD_{50} of intraperitoneally injected pentylenetetrazol was determined. Aspartame treatment produced large increases in plasma and brain phenylalanine and tyrosine, but did not alter the CD_{50} of pentylenetetrazol either in GEPR-3s or GEPR-9s.

6.1.2.3 Electroshock seizures

The effects of orally administered bolus doses of aspartame on electroshock convulsions in GEPR-3s and GEPR-9s were investigated by Jobe and colleagues (1992a). Tonic extensor convulsions induced via corneal electroshock were assessed for effects of aspartame doses between 50 and 3000 mg/kg. This type of seizure is driven by brainstem circuitry (Browning, 1987b). The convulsions begin with tonic flexion of the pelvic limbs and progress to tonic extension. The ratio of the duration of extension divided by the duration of flexion provides an index of seizure severity (Swinyard, 1972). The magnitude of the ratio is directly related to the degree of seizure severity. In our experiment with aspartame, no alterations occurred in mean extension/flexion ratios in response to the sweetener. Moreover, linear regression analysis of individual values of extension/flexion ratios and amino acids provided no evidence of aspartame-induced seizure facilitation. The absence of a proconvulsant effect due to aspartame occurred despite large increases in plasma phenylalanine and tyrosine concentrations and transport ratios and despite significant decrements in tryptophan transport ratios.

6.1.3 Epileptic baboons

The epileptic baboon, *Papio papio*, represents a primate model of human epilepsy (see Jobe et al., 1992b for a review). Epilepsy predisposition in these animals includes susceptibility to seizures induced by an intermittent light stimulus (Killam et al., 1966a,b; 1967). Investigators have observed that seizures can also be induced in the epileptic baboon by hyperventilation, excessive exertion, stress and restraint. Moreover, the threshold for pentylenetetrazol-induced seizures appears to be abnormally low. Spontaneous seizures have also been reported to occur (Killam, 1976; Killam and Killam, 1984; Killam et al., 1967).

The search for aspartame-induced effects on seizures has been undertaken in the epileptic baboon by Meldrum and coworkers (1989). Assessments of possible changes in seizure susceptibility and severity were undertaken. Aspartame was administered in single oral doses of 300 or 1000 mg/kg. Animals treated with tap water served as controls. No effects on seizure indices were forthcoming at either dose. Baboons treated with tap water had plasma phenylalanine and tyrosine levels of approximately 55 and 38 nmol/ml, respectively. Following the aspartame dose of 1000 mg/kg the concentrations rose to approximately 2000 and 85 nmol/ml, respectively. The phenylalanine transport ratio was approximately 0.14 in tap water treated baboons. A value of 3.83 was reached 2 hours following 1000 mg/kg of aspartame. The tyrosine transport ratio actually fell below pretreatment levels when the animals were treated with the 1000 mg/kg dose of aspartame. Also, the phenylalanine/tyrosine ratio rose from the 1.39 of baboons treated with tap water to 24.7 in animals treated with the large dose of aspartame. Based on these data, Meldrum and colleagues (1989) concluded that there is no evidence for a proconvulsant action of high doses of aspartame in primates.

6.2 Studies in non-epileptic animals

Neurologically normal mammals will exhibit seizures in response to convulsant drugs and electrical stimuli (Dreisbach, 1983; Swinyard, 1972; Ward, 1972; Stone, 1972). Moreover, normal mammals will "kindle" in response to low level electrical stimuli (Corcoran, 1988; Goddard, 1967; Goddard et al., 1969; McNamara et al., 1980, 1985; Racine, 1972, 1978; Racine et al., 1972). A small current delivered at appropriate intervals into specific nuclei of the brain produces increasingly greater responses. Initially, convulsive motor activity does not occur. But, with repetition, seizures cause increasingly severe convulsions. Eventually, a "fully kindled" state characterized by predictable convulsive behavior is achieved.

Attempts to identify effects of aspartame on seizures in normal animals have been predicated upon the concept that convulsant and anticonvulsant drugs influence seizures in non-epileptic animals essentially in a way that corresponds with their influence on seizures in non-epileptic humans. Accordingly, drugs which prevent seizures in humans (Dreisbach, 1983; Rall and Schleifer, 1990) exposed to convulsant drug overdoses, also suppress seizures in non-epileptic animals exposed to convulsant stimuli. Moreover, drugs which are convulsant in non-epileptic humans (Dreisbach, 1983; Rall and Schleifer, 1990) are also convulsant in other normal mammals (Stone, 1972).

6.2.1 Pentylenetetrazol-induced seizures

Several groups have examined the effects of aspartame on pentylenetetrazol-induced seizures in non-epileptic rodents. Results have been mixed in that two groups reported that large aspartame doses caused modest facilitation of seizure expression in rats (Guiso et al., 1988) and in mice (Pinto and Maher, 1988) while others were unable to detect facilitation (Nevins et al., 1986a,b; Dailey et al.,

1988; 1989a; Tilson et al., 1989). These findings have been reviewed previously (Sze, 1989; Fisher, 1989).

Earlier studies showed that aspartame doses up to 500 mg/kg did not facilitate pentylenetetrazol-induced seizures in fasted mice that ranged in weight from 20–28 g (Nevins et al., 1986a,b). Pinto and Maher (1988) reported that oral aspartame doses of 1000 to 2000 mg/kg facilitated pentylenetetrazol-induced seizures in immature CD-1 mice (10–13 g) that had not been fasted. They also found that equimolar doses of phenylalanine were similarly effective. Dailey and colleagues (1989a) working with CD-1 mice attempted to replicate the findings of Pinto and Maher (1988) but were unsuccessful. As part of the attempts at replication of the Pinto and Maher (1988) data, Dailey et al. (1989a) administered doses of aspartame as high as 2500 mg/kg. Aspartame, by gavage, was given to immature mice (9–13 g), to more mature animals (24–32 g), to mice that were fasted and caged individually, to mice that were fasted and caged in groups, and to mice that were fed and caged either individually or in groups. None of the treatments caused facilitation of pentylenetetrazol-induced seizures in CD-1 mice.

Guiso et al. (1988) reported that aspartame (750 or 1000 mg/kg) given orally to fasted rats significantly increased the number of pentylenetetrazol-induced clonic-tonic seizures. A similar effect was found for equimolar doses of phenylalanine. The authors described these effects as "weak." Also, when aspartame was given to fasted rats in three divided doses (330 mg/kg × 3) over 120 minutes, or was given to animals after they had eaten, or was given to animals in their diet, no effects on seizures were detected.

Dailey et al. (1988) were unable to detect aspartame-induced facilitation of pentylenetetrazol-induced seizures in fasted GEPRs or in fasted seizure resistant Sprague-Dawley derived rats even with acute aspartame doses as high as 2000 mg/kg. Similarly, Tilson et al. (1989) found that 1000 mg/kg of aspartame by gavage had no effect on pentylenetetrazol-induced seizures in fed or fasted Fisher-344 rats.

The reasons for the divergent findings summarized above are not entirely clear although some methodological issues have been discussed in this regard (Dailey et al., 1989a; Tilson et al., 1989). At any rate, these studies do not provide clear evidence of a proconvulsant effect of aspartame in pentylenetetrazol-induced seizures in rodents.

6.2.2 Bicuculline-induced seizures

Again, in the same abstract previously cited in Sections 6.1.1.1 through 6.1.1.3, Chiu and Woodbury (1988) reported that aspartame had effects on bicuculline-induced seizures in C57 mice. At doses of aspartame "below 100 mg/kg" the CD_{50} for bicuculline was said to be "decreased slightly." As noted previously (paragraph 6.1.1), abstracts are inherently brief and the data summarized in this abstract were not presented at the meeting.

In our laboratory, aspartame did not facilitate bicuculline-induced seizures in C57 mice (Dailey et al., 1989c). In our study, aspartame doses (10, 30, 300, and 1000 mg/kg) were administered by gavage to C57 male mice. Four hours

after the aspartame or vehicle gavage all mice received a CD_{50} dose of bicuculline. No aspartame dose was associated with an increase in the incidence or severity of bicuculline-induced seizures.

6.2.3 Lidocaine-induced seizures

The effects of aspartame on seizures induced by lidocaine in adult CD-1 mice have been evaluated by Kim and colleagues (1988). Orally administered aspartame in doses up to 600 mg/kg had no effect on the CD_{50} of lidocaine. In contrast, statistically significant decrements in the CD_{50} were reported to occur when aspartame was given intraperitoneally in single bolus doses as low as 50 mg/kg. The time interval between the administration of aspartame and lidocaine was not reported.

The reported facilitation of lidocaine-induced seizures by intraperitoneally administered aspartame is not relevant to the dietary use of this sweetener. In the human diet, aspartame is taken orally not intraperitoneally. Other experimental observations show that the intact aspartame molecule itself is not absorbed from the gastrointestinal track into the blood (Ranney et al., 1976). The aspartame molecule per se may have some direct or indirect effects on seizures when given by the intraperitoneal route. The observations of Kim and coworkers (1988) show that these effects are absent when aspartame is given orally.

6.2.4 Electroshock seizures

In the abstract first cited in Section 6.1.1.1, Chiu and Woodbury (1988) described preliminary observations pertaining to aspartame and electroshock seizures in non-epileptic C57 mice. According to their report, a single aspartame oral dose of 200 mg/kg both facilitated and suppressed minimal electroshock seizures. They found the threshold to be increased at 0.5 hours and decreased at 4 hours. The details of these experiments have not been published in the form of a manuscript.

Since multiple lines of evidence support the concept that seizures caused by minimal electroshock are driven by forebrain circuitry (Browning, 1987b, 1991), the preliminary results of Chiu and Woodbury might be an indication that aspartame influences forebrain seizures. The observations of Tilson and colleagues (1989) tested this hypothesis by employing limbic seizures. But, electrical kindling of forebrain limbic structures occurred at the same rate in rats treated with 1000 mg/kg of aspartame as they did in animals treated with saline. In both cases, the fully kindled state was achieved upon delivery of the 18th electrical stimulus to the deep prepyriform cortex.

Electroshock and aspartame studies in non-epileptic animals have not been limited to tests of effects in forebrain seizures. Brainstem seizures have also been investigated (Guiso et al., 1988; Jobe et al., 1992a; Nevins et al., 1986a,b; Tilson et al., 1989). Electrically-induced tonic extensor convulsions in rats have been employed as convenient indices of this type of seizure. The evidence supporting the concept that these seizures, also called maximal electroshock convulsions,

are an index of brainstem seizure activity has been summarized by Browning (1987b, 1991).

In each aspartame assessment, the orally administered sweetener failed to alter maximal electroshock seizures. In some of the studies, doses as high as 3000 mg/kg were utilized. Nevins and colleagues (1986b) found that when given by gavage 30 minutes prior to tests for seizure endpoint, aspartame doses of 0, 50 and 500 mg/kg failed to alter the CC_{50} (convulsive current in 50% of the animals) for tonic extensor convulsions in CD-1 mice. Guiso and colleagues (1988) reported that aspartame in a dose of 1000 mg/kg failed to exert a significant effect on the CC_{50} for tonic extensor convulsions in rats. Using the percentage of rats exhibiting tonic convulsion as their endpoint, Tilson and coworkers (1989) showed that aspartame given by gavage did not enhance the response compared to control rats given saline by the same route of administration. Jobe and colleagues (1992a) used the extension/flexion ratio as an index of seizure severity. Previous work had shown that an experimentally-induced decrease in noradrenergic activity results in an increase in the ratio (Browning and Maynert, 1978), an effect that is opposite that of antiepileptic drugs. But administration of aspartame in doses as high as 3000 mg/kg had no effect on the extension/flexion ratio in non-epileptic rats. Interestingly, the absence of effects on electroshock seizures occurred despite marked increases in plasma phenylalanine and tyrosine concentrations (Guiso et al., 1988; Jobe et al., 1992a) and transport ratios (Jobe et al., 1992a) which occurred in response to the oral administration of aspartame. Moreover, the absence of a proconvulsant effect also occurred despite aspartame-induced decreases in the tryptophan transport ratios (Jobe et al., 1992a).

6.2.5 In vitro seizure activity

Fountain and coworkers (1988) evaluated the effects of aspartame on the excitability of the rat hippocampal slice. Aspartame containing solutions were applied directly to the slice preparation in concentrations of 10, 100, 1000 and 10,000 nmol/ml. All aspartame doses significantly potentiated the population spike responses of CA1 pyramidal cells when comparisons were made to responses characteristic of slices maintained in the pretreatment incubation medium. Moreover, application of a 100 nmol/ml solution of phenylalanine to the slice produced excitatory effects.

How are these observed excitatory responses to be interpreted? We suggest that two major issues are especially pertinent to the process of determining the significance of the data: (1) the aspartame moiety was applied directly to the hippocampal slice; and (2) treatment effects were compared to the responses of the slice preparation incubated with solutions vastly different than those that bathe the intact hippocampus.

With regard to the first issue, the direct application of aspartame to the *in vitro* hippocampus exposes this tissue to a chemical moiety that does not have access to the *in vivo* hippocampus when aspartame is used as a sweetener in human diets. Accordingly, orally ingested aspartame is degraded to phenylalanine, aspartic acid and methanol before it is absorbed into the blood (Ranney

et al., 1976). Therefore, effects of the aspartame moiety *per se* on the hippocampus are not pertinent to dietary questions.

However, Fountain et al. (1988) also found excitatory effects when phenylalanine was directly applied to the hippocampus. It might, therefore, be argued that the effects of direct phenylalanine application are relevant to the dietary use of aspartame. Indeed, phenylalanine derived from aspartame is systemically absorbed and it crosses the blood/brain barrier. But, interpretation of the phenylalanine-induced excitatory effects reported by Fountain and colleagues (1988) requires a consideration of our second major issue; namely, that treatment effects in the slice preparation were compared to the responses of the slice incubated with solutions qualitatively different than those that bathe the intact hippocampus. Accordingly, prior to the application of the test solution containing phenylalanine, the slices were incubated in a medium of NaCl, KCl, $MgSO_4$, $CaCl_2$, $NaHCO_3$, glucose, O_2, and CO_2. We find it notable that this pretreatment solution contained no phenylalanine. Indeed, this control preparation was devoid of any amino acids that would be found in the extracellular fluid of the intact hippocampus or any other *in situ* brain structure. Unpublished observations from our laboratory using intracerebral microdialysis show that extracellular fluid of the *in situ* striatum contains phenylalanine in concentrations of approximately 5 to 20 nmol/ml in unanesthetized, unrestrained and untreated rats. Moreover, we found that, following the administration of aspartame (2000 mg/kg orally), phenylalanine concentrations peak at approximately 80 nmol/ml.

Since normal extracellular fluid contains phenylalanine and the control solution used by Fountain and coworkers (1988) did not, their data do not reveal whether the only concentration of this amino acid (100 nmol/ml) used in their experiments produces a level of responsiveness greater than would result in the presence of a solution containing normal concentrations (5 to 20 nmol/ml). However, even if the responses were in reality greater, dietary relevance would still be lacking. Their 100 nmol/ml concentration is greater than the peak 80 nmol/ml concentration occurring in extracellular fluid of *in situ* brain structures following an oral aspartame dose of 2000 mg/kg. Doses of 2000 mg/kg are approximately 800 times greater than the 90th percentile average daily consumption of aspartame in humans (Butchko and Kotsonis, 1991). This line of reasoning even more emphatically precludes imputing dietary relevance to the excitatory effects of the 1000 and 10,000 nmol/ml solutions of aspartame which were applied by Fountain and colleagues to the hippocampal slice.

6.2.6 Flurothyl-induced seizures

Pinto and Maher (1988) reported that oral aspartame doses of 1000, 1500 and 2000 mg/kg decrease the latency to the onset of clonic convulsions produced by flurothyl in CD-1 mice. These investigators reported in the same manuscript that these doses of aspartame also enhanced pentylenetetrazol-induced seizures in CD-1 mice. But, as set forth in Section 6.2.1 above, replication of their findings by other investigators has not been possible despite use of the same pentylenetetrazol CD-1 mouse model and despite the use of doses equal to (1500 and 2000 mg/kg) or a higher dose (2500 mg/kg) of aspartame (Dailey et al., 1989a).

6.2.7 Kindled seizures

Tilson and coworkers (1989) observed that, in adult Fischer-344 rats, aspartame given by gavage had no effect on the rate of electrically-induced limbic kindling. Three treatment protocols were employed, but in each one, every dose of aspartame was 1000 mg/kg. In the first protocol, only two aspartame doses were given, the first being 2 hours before the initial kindling stimulus was delivered and the second being 6 hours after the first dose. Kindling stimuli were delivered every hour for a total of 18 hours. Both in the aspartame treated group and the saline treated group, the fully kindled stage (class 5 convulsion) was reached at the same rate of development.

In the second paradigm, aspartame doses were given twice daily every day with the last two doses being given on the 14th day. Kindling stimuli were initiated on day 15 at the rate of one per hour. At the end of the 20th stimulus, the rate of epileptogenesis in the aspartame group equaled that of the saline group.

In the third paradigm, aspartame was given twice daily for 14 days as it was in the second protocol. However, kindling stimuli were applied two hours after each aspartame dose beginning with the first dose. The electrical stimuli were applied at intervals of twice daily and continued for 18 days. No significant differences in kindling responses were detected between the aspartame and saline treated groups. Based on the observations in all three treatment paradigms, the authors concluded that aspartame does not facilitate electrical limbic kindling in rats.

Another carefully designed kindling study was undertaken by Cain and coworkers (1989). Both amygdala and hippocampal kindled seizures were assessed. Aspartame-induced facilitation of kindling did not occur despite the administration of single acute oral doses from 25 to 2000 mg/kg.

6.2.8 Quinolinic acid-induced seizures

The absence of effects of orally administered aspartame on seizures induced by the excitatory amino acid quinolinic acid has been reported by Guiso and colleagues (1988). These investigators administered aspartame to fasted rats in a dose of 1000 mg/kg 60 minutes before the administration of 120 nmol of quinolinic acid into the hippocampus. When compared to vehicle given by gavage, neither the incidence of seizures or the total time spent in seizures changed in response to aspartame administration. Also, the authors reported that the effect of a threshold dose of the quinolinic acid (60 nmol) was also unaltered by pretreatment with the sweetener.

6.2.9 Theophylline-induced seizures

A study was carried out in order to determine if oral aspartame would influence seizures produced by theophylline. Zhi and Levy (1989) administered aspartame, 1000 mg/kg, or an equimolar dose of phenylalanine to fasted female Lewis rats. One hour later, theophylline (40 mg/ml as aminophylline) was infused at a

constant rate through an indwelling jugular cannula which had been implanted two days previously. The infusion was continued until the rats had a "maximal seizure". Neither aspartame nor phenylalanine altered the dose of theophylline that was associated with seizures or the time that it took to produce seizures. Also, the theophylline concentration in serum and cerebrospinal fluid at the time of seizures were virtually identical among controls and aspartame or phenylalanine treated animals. Thus, these results not only show that aspartame and phenylalanine do not alter seizures induced by theophylline in rats but also suggest that the concomitant use of aspartame and a methylxanthine does not enhance the seizure potential of the methylxanthine.

6.3 Studies in humans

Speculations that aspartame causes or facilitates seizures in humans have appeared both in the scientific literature and in the popular press. Additionally, some publications describe isolated uncontrolled, anecdotal observations of seizures in people also ingesting aspartame. In addition, prospective studies are underway and preliminary results have become available in the literature.

With respect to human use of aspartame and its purported liability for inducing seizures, many relevant studies have been carried out to evaluate the metabolism and pharmacokinetics of aspartame and its components in animals and humans. Aspartame is metabolized by esterases and peptidases in the gastrointestinal tract to three naturally occurring dietary components – aspartate, phenylalanine and methanol. The amounts of these components are small compared with the quantities obtained from common dietary sources.

When 34 mg/kg of aspartame is given as a bolus to healthy adults, the plasma concentrations of aspartate and methanol are unchanged from the fasting baseline. Although plasma phenylalanine concentration rises significantly after such a large bolus dose of aspartame, the peak concentration remains within the normal postprandial range (Stegink et al., 1977, 1981). Thus, bolus doses of aspartame approximately 14 times the 90th percentile daily consumption levels result in plasma concentrations of aspartame's components that are in the normal range. Furthermore, when large repeated doses of aspartame are given every hour for eight hours (a total of approximately 32 times current 90th percentile average daily consumption), plasma phenylalanine concentrations approximate the normal postprandial range (Stegink et al., 1989; 1990).

In addition, as discussed in Section 4.0, any effect that the phenylalanine from aspartame may have on the Phe/LNAA ratio, the mechanism by which aspartame has been hypothesized to affect brain neurotransmission and consequently seizure threshold is not unique to aspartame. Ingestion of equivalent sweetness amounts of sucrose leads to changes in this ratio of similar magnitude through insulin-mediated reductions in the plasma concentrations of some of the amino acids which make up the denominator of the ratio (Martin-Du Pan et al., 1982; Burns et al., 1991; Wolf-Novak et al., 1990). Furthermore, as described in Section 4.0, even enormous doses of aspartame, up to approximately 1000 times the 90th percentile aspartame consumption do not affect seizures in animals, including primates.

The results of studies evaluating the effects of aspartame on seizures in humans are summarized below.

6.3.1 Anecdotal cases of Wurtman (1985)

Wurtman (1985) presented three people who experienced seizures and also ingested aspartame from dietary sources. Each experienced a tonic-clonic seizure. All were adults. Wurtman (1985) concluded that a causal relationship between aspartame ingestion and the occurrence of seizures could not be established on the basis of his data. But, based on neurochemical and neuropharmacologic relationships established in our laboratory (Jobe et al., 1984), Wurtman cautioned that a causal relationship might exist because high aspartame doses may produce neurochemical changes that in laboratory animals are associated with seizure facilitation. This precautionary note by Wurtman sparked the diverse battery of experiments described in Sections 6.1 and 6.2 above. The weight of evidence derived from these studies shows that the aspartame-induced seizure-facilitating neurochemical effects envisioned by Wurtman do not occur even when the sweetener is administered in doses beyond those needed to approximate amino acid changes in plasma associated with the dietary use of aspartame or even with abuse doses of aspartame in humans.

6.3.2 Single case report by Walton (1986)

Walton (1986) described a 54-year old woman with unipolar affective disorder who experienced a grand mal seizure approximately five years after maintenance therapy with the antidepressant drug imipramine was initiated. Following the seizure, she was hospitalized and noted to be experiencing euphoria. A psychiatric consultation detected psychomotor acceleration, flight of ideas and grandiosity. The imipramine therapy was discontinued at this point and the patient was discharged from the hospital. Continued mania at home led to re-hospitalization and the administration of lithium carbonate, an antimania medication. After discharge, imipramine therapy was reinstituted.

Reportedly, the patient had a history of consuming large amounts of iced tea. During the summer, the daily intake approached one gallon. During the several weeks before the seizure and the onset of mania, the patient had sweetened her iced tea with aspartame. During the second hospitalization, the aspartame was replaced by sugar and the patient continued to consume large amounts of tea. Seizures were not observed subsequent to discharge from the second sojourn in the hospital. But, neither were they observed during the first hospitalization or during the interim between that time and the onset of the second hospitalization.

The report of Walton (1986) describes only one patient, lacks valid means to control for multifaceted environmental and innate influences other than aspartame ingestion, and lacks the crucial element of randomness in patient selection. Even though these authors selected their own methodology, published options exist to assist decision making even with single patient trials. Rigorous

methodology for single-patient clinical trials was developed earlier (Kazdin, 1982) and applied to clinical medicine (Frasca and Aldag, 1988; Guyatt et al., 1986; Porta, 1986; McLeod et al., 1986). Interestingly, Frasca and Aldag (1988) applied the rigorous design of the single patient clinical trial to the question of aspartame-induced headache in one of their patients. Their analysis included the use of aspartame containing capsules (given in a dose of 300 mg three times daily) placebo capsules, four crossovers lasting two weeks each, a two-week baseline period, a washout interval of one week between each crossover and a randomized design for sequencing placebo and aspartame trials. The results of their study were analyzed with the Cochrane Q test and repeated measures analysis of variance. No statistically significant association existed between aspartame ingestion and the occurrence, duration or severity of the patient's headaches.

6.3.3 Retrospective descriptions of aspartame ingestion in people who also experience seizures: a report by Roberts (1988)

Roberts (1988) reported in an abstract that 95 people experienced grand mal, petit mal or psychomotor seizures while consuming aspartame. A paucity of experimental detail is provided. A scientific basis for imputing a causative role to aspartame is not set forth. Nevertheless, the author arrives at this conclusion. No quantitative dose response or time-course findings are presented. No control group is described. No means are presented for determining whether seizures occurred more frequently in patients taking aspartame than in patients not taking aspartame. We are confident that many clinics have seen 95 or more patients that experienced seizures when they have taken aspartame and again when they have not. People with epilepsy commonly consume aspartame. The segment of the human population with epilepsy is at high risk for seizures. We would be surprised if epilepsy patients ingesting aspartame were to stop having seizures after ingestion of the sweetener.

The author also mentions that the contributory role of aspartame was supported by "gratifying improvement or disappearance of seizures after its cessation." Moreover, they point out that recurrence of attacks was "prompt and predictable" upon aspartame rechallenge – even in small amounts (e.g. chewing gum)." No data are provided to compare the incidence of seizures during abstinence from aspartame with the incidence during rechallenge. How much time elapsed between withdrawal and rechallenge?

Finally, the author attributes the "possible epileptogenic or co-epileptogenic" effect of aspartame to various mechanisms including "altered neurotransmitter function due to phenylalanine excess, the effects of methyl alcohol." These ideas are not credible in view of the experimental data that are now available showing the lack of effect on neurotransmitters. Also, the extremely small amount of methanol that might be ingested with a stick of chewing gum is far below the content of a glass of fruit juice and much less than could produce a measurable change in plasma methanol concentration (see Section 3.0).

6.3.4 Prospective, randomized, double-blind, placebo-controlled crossover study of aspartame in individuals who had seizures allegedly related to aspartame: an investigation by Rowan and Shaywitz (1992)

An abstract describing some of the results of a study by Rowan and Shaywitz (1992) was presented at the annual meeting of the American Electroencephalographic Society in Philadelphia in December 1991. These investigators evaluated the effect of aspartame on the incidence of seizures or electroencephalographic discharges in eighteen patients who reportedly had seizures as a result of aspartame consumption. Upon entering the experimental protocol, each person received on different days, aspartame or placebo via a capsule ingested orally. Aspartame was administered at the rate of 50 mg/kg daily in equally divided doses given at 08:00, 10:00, and 12:00 hours in a randomized, double-blind, placebo-controlled, crossover design. The protocol used by the investigators employed a period of six in-patient days: (Day 1) a screening day; (Days 2, 4, and 6) baseline days; and (Days 3 and 5) treatment days. During these days neurological examinations, continuous electroencephalographic monitoring and determinations of plasma amino acid concentrations were undertaken. Upon evaluation of the results, the authors found that no seizures occurred following aspartame administration. Electroencephalographic seizures were noted in two patients following placebo ingestion. According to the presentation, four patients in the study had a diagnosis of absence seizures. Despite the aspartame treatment, none of these four individuals exhibited clinical seizures. Rowan and Shaywitz interpreted their results to indicate that aspartame consumption is no more likely than placebo to provoke seizures in subjects who reportedly experienced seizures related to aspartame consumption prior to entering their randomized, double-blind, placebo-controlled, crossover study.

6.3.5 A prospective, crossover, controlled study of aspartame in patients with absence seizures: a study by Camfield and colleagues (1992)

Ten children with newly diagnosed and untreated absence seizures were evaluated by Camfield and colleagues (1992) for responses to aspartame. Ambulatory EEG recordings were employed to quantify spike-wave discharges in a double-blind, crossover experiment. The patients randomly received either 40 mg/kg of aspartame or a sucrose sweetened drink. The investigators evaluated the number of spike-wave bursts per hour rather than the occurrence of clinical seizures. Of the EEG parameters evaluated, i.e., number of spike-wave bursts per hour, duration of spike-wave bursts, and the number of seconds per hour spent in spike-wave discharges, a single statistically significant difference between aspartame and sucrose treatment arms was found. This difference was in the mean number of seconds per hour of EEG that the children spent in spike-wave discharges and was higher following aspartame ingestion.

These studies are intriguing. They are compatible with the hypothesis that aspartame facilitates spike-wave discharges. They are also compatible with other interpretations. For example, they are consistent with the possibility that sucrose or the sensory stimulus associated with the taste of sucrose affects the EEG,

including spike-wave discharges detected therein, differently than aspartame. Fishbein and colleagues (1990) have already noted that glucose affects the EEG. The early observations of Gibbs and colleagues (1939) suggest that blood sugar levels inversely affect spike-wave discharges of absence seizures. Accordingly, high blood sugar concentrations protected their patients against 3 per second spike-wave activity. Thus, it is possible that the use of sucrose as a control may have introduced an additional variable into the study results. The lack of an inert placebo control makes it impossible to assess if any observed differences in spike-wave discharges between aspartame and sucrose treatments were not, in fact, due to a decrease in spike-wave discharges during sucrose treatment rather than an increase during aspartame treatment. In addition, given the variability of the occurrence of spike-wave discharges (Guey et al., 1969) and the absence of a comparable untreated period of EEG recording, it may not be possible to determine precisely if the observed change is clinically relevant.

Camfield and colleagues noted that the amount of spike-wave discharge on EEG does not translate directly into absence seizure severity and children with absence epilepsy often show bursts of spike-wave on EEG when clinical absence attacks are not apparent. Therefore, although there was a statistically significant difference in the number of seconds/hour occupied by spike-wave discharges found in this study, it does not appear to translate into an effect on clinical absence seizures. Even in a well-designed initial clinical trial, there are many variables that cannot be completely controlled. Thus, establishment of an association between aspartame use and absence seizure severity awaits verification in experimental models of absence seizures and in large scale clinical trials.

6.3.6 Prospective, randomized, double-blind, placebo-controlled crossover study of aspartame in PKU heterozygotes: an investigation by Benninger and colleagues (1991)

These workers evaluated the electroencephalographic effects of chronic aspartame administration in 48 PKU heterozygotes who received either 15 or 45 mg/kg of aspartame/day in capsules for 12 weeks and placebo capsules for 12 weeks. They found no significant differences in the clinical EEG evaluation or in any of the EEG spectral parameters when data from aspartame treated subjects were compared to those with placebo treatment. They interpreted their data as a reaffirmation of the safety of aspartame.

6.3.7 Prospective, randomized, double-blind, placebo-controlled, crossover study to evaluate the effect of aspartame in children with documented seizure disorders: a study by Shaywitz and colleagues (1992)

A study evaluating the effects of aspartame in nine children with documented seizure disorders was recently published in abstract form. This study was a randomized, double-blind, placebo-controlled, crossover study. Six of the children had generalized convulsions, four of these also had absence seizures. One child had absence seizures only and two children had complex partial seizures only. The children received 34 mg/kg of aspartame or placebo in capsules every

day for two weeks, at which time they crossed over to the other treatment. During each treatment arm, the children were admitted to a clinical research unit where both standard 21-lead EEG and continuous 24-hour EEG were performed. No clinical seizures were noted during the study. EEG's were similar in both treatment arms for 8 of the children. One child had a more abnormal EEG during placebo treatment. The investigators concluded that large doses of aspartame do not provoke seizures or increase epileptiform discharges in vulnerable children.

6.3.8 Additional clinical considerations

Fisher (1989) points out that approximately 100 million Americans regularly consume products containing aspartame. Five million Americans have had at least one seizure. According to his assumptions and calculations more than 1 million American people with seizures also consume aspartame. Fisher (1989) reminds us that people with seizure disorders are at high risk for seizures. We should not, therefore, be surprised that some of them experience seizures after consuming aspartame even if this substance does not facilitate seizures.

Fisher (1989) also makes another point that warrants consideration: "The Epilepsy Institute of New York has noted no change in seizure pattern, since introduction of aspartame, among their 5,000 client visits per year." If significant seizure liability were a property of aspartame and if epilepsy patients were especially susceptible to such an effect, clinics with large patient populations would be a likely place to detect the effect. The absence of an increase in the Epilepsy Institute of New York is compatible with the concept that aspartame does not have seizure facilitating effects or that these effects are too small to detect even in large groups of people subjected to a multiplicity of variables.

Finally, Bradstock and colleagues (1986), working with data received by the Division of Nutrition of the Center for Disease Control, and Tollefson and coworkers (1988), working with data received by the U.S. Food and Drug Administration, formally evaluated consumer complaints associated with aspartame consumption. The report for the Center for Disease Control found no symptom complex suggestive of a widespread public health hazard associated with aspartame use. The report for the Food and Drug Administration evaluated complaints of seizures, headaches, memory loss and several other reported symptoms. They concluded that, "There is currently no consistent or unique pattern of symptoms reported with respect to aspartame that can be causally linked to its use."

7 The issues in overview

7.1 Hypothetical concerns: neurochemical effects of aspartame

The original concerns that aspartame might facilitate convulsive seizures were founded on neurochemical mechanisms of seizure regulation. Aspartame-induced decrements in noradrenergic and serotonergic activity of the brain were originally believed to occur. Subsequent experimentation did not substantiate

the noradrenergic part of the hypothesis. Moreover, aspartame-induced reductions in serotonin stores were too small to facilitate seizures and they occurred only in doses of the sweetener that were beyond those that might be ingested for dietary purposes in humans.

7.2 Hypothetical concerns: transport ratios and tyrosine/phenylalanine ratios

Some authors have asserted that the dietary consequences of aspartame in humans can be mimicked in rodents only if doses of aspartame are appropriately chosen. According to their reasoning, the dose of aspartame in rodents must be 60 fold higher than in humans. This line of reasoning led the proposition that a single bolus dose in rats and mice should be as high 3000 mg/kg. The rationale that led to this idea was predicated upon the fact that rodents have liver phenylalanine hydroxylase activity which is 5 fold higher than do humans. According to this concept, the greater amount of tyrosine formed from phenylalanine in rodents serves as an "antidote" to the effects one might expect from phenylalanine. However, Hjelle et al. (1992) have demonstrated that rodents require only 2 to 6 times more aspartame than humans in order to attain similar Phe/LNAA ratios. Thus, although studies in animals were done with doses of aspartame up to 3000 mg/kg, doses of aspartame as low as 4 to 18 mg/kg in rodents would be equivalent to current human exposure (Butchko and Kotsonis, 1991).

The experiments that were carried out in response to the high dose hypothesis, have shown that convulsions are not facilitated or caused by single oral doses as high as 3000 mg/kg. Lack of seizure facilitation was evident despite the significant elevations in phenylalanine transport ratios and despite the increases in phenylalanine/tyrosine ratios that mimicked those of abuse doses in humans.

7.3 Protracted dosing

Both subacute and subchronic studies of orally administered aspartame have been undertaken. The subacute investigation utilized normal rats subjected to electrical kindling and the subchronic inquiry employed genetically epileptic rats. The daily doses in these experiments ranged from approximately 40 mg/kg to 2000 mg/kg. Aspartame-induced effects on forebrain and brainstem seizure indices were not detected in either of the two types of experimentation.

A chronic study of 12 weeks of oral aspartame administration (15 or 45 mg/kg/day) was conducted in humans. The resulting EEGs did not differ from those of placebo treatment.

7.4 Experimental design: uncontrolled variables

A few initial investigations in rodent seizure models reported aspartame-induced facilitation of convulsions. But in those instances where other laboratories have worked to replicate the effects, no proconvulsive properties have been identified. Failure to replicate has emerged despite the use of the same strain, sexes and ages of animals, the same doses of aspartame and the same time intervals

between dosing and seizure testing as were used in the original studies. Controls for these key variables accounted for many commonly known influences that otherwise might also have inadvertently led investigators to assign inappropriate significant effects to aspartame. However, the initial studies appeared not to have controlled for other key pitfalls including specific procedural, temporal and environmental variables. Loscher and colleagues (1990, 1991a,b) have provided useful insights into these occasionally uncontrolled influences. Moreover, they have illustrated how such influences cause different groups of researchers to report divergent findings on the same test compounds evaluated at comparable or identical doses in the same model and species.

Specifically, some partially uncontrolled reports of aspartame-induced seizure facilitation have compared the effect of a single vehicle treated group with the effects on seizure indices of many different administrations of test substance, including those given in single and multiple dose paradigms. Such designs probably do not account for the effects on seizures produced by different types of handling stress and injection routes. The designs have also used large numbers of animals accumulated because of the diverse treatments. Unfortunately, many laboratories do not have the facilities or resources to make the comparisons all in one afternoon. Some experiments are so large that comparisons would likely have been made across days, weeks or months. Moreover, the methods sections of the papers do not describe a means for addressing the variables that are seemingly uncontrolled.

Pentylenetetrazol thresholds have been noted to vary considerably, even when determined consecutively within one week, even in the absence of treatment with drugs that might alter seizure responses (Loscher et al., 1991b). Also, temporally related variations in electroshock seizures have been noted by Porszasz and Worum (1971). Our own investigations with pentylenetetrazol-induced convulsions in CD-1 mice revealed notable variations in the CD_{50} in vehicle-treated groups tested in different months (Dailey et al., 1989a).

Because of the susceptibility of seizure indices to circadian, seasonal, environmental and procedural variables, experimentation must be rigorously controlled and designed. Loscher and colleagues (1991b) have found that the effects of drugs on pentylenetetrazol-induced seizure thresholds must be determined within the same time-frame and under the exact conditions used for control thresholds. Similar restrictions must also be applied to the use of electrically-induced seizures which are known to vary temporally (Porszasz and Worum, 1971). In the absence of such controls, differences may be detected and attributed to treatment, when they are actually due to other uncontrolled variables. The methods sections of some reports showing no effects of aspartame on seizure indices stipulate that specific procedures were used to control for temporal, environmental and procedural variables described above. For example, in the study by Meldrum and colleagues (1989), all of the epileptic baboons used in the experiment received aspartame doses of 0, 300 or 1000 mg/kg in random order (repeated Latin squares design) at weekly intervals. As another example, in our experiments with aspartame (e.g. Dailey et al., 1989a, 1991; Jobe et al., 1992a), each treatment group was matched with a control group so as to eliminate extraneous variables as potential causes of differences between treatments and

controls or between different doses of the treatment. With our approach, the matching process must include: (1) random distribution of animals into treatment and control groups from the same pool of animals once the pools has been received from the supplier; (2) experimental processing of the control and aspartame groups in a temporally interdigitated manner; and (3) observation for convulsive endpoints in the same temporally interdigitated fashion.

7.5 Aspartame administration paradigms that bypass the gastrointestinal tract

In some instances, seizure facilitating effects were detected when aspartame molecules were placed into direct contact with brain tissue or when the molecules were administered intraperitoneally. These effects lack relevance to the dietary use of aspartame. When consumed as a sweetener, aspartame is converted to phenylalanine, aspartic acid and methanol within the gastrointestinal tract. These latter moieties rather than aspartame itself are absorbed into the blood. In one instance where the hippocampal slice was used as an *in vitro* seizure model, phenylalanine produced excitatory effects when applied to the preparation. But, these effects were relative to the lower level of inherent excitation of a slice maintained in the absence of amino acids, including phenylalanine, in the incubation medium. Since normal extracellular fluid of the brain contains phenylalanine, the normal excitatory state of the hippocampal slice should be more closely mimicked by a solution containing the amino acid than one in which it is absent.

7.6 Aspartame and convulsive seizures

The bulk of the experimental evidence supports the concept that orally administered aspartame does not cause or facilitate convulsive seizures in either epileptic or non-epileptic mammals including humans. Proconvulsive effects appear to be absent both in seizures arising from forebrain and from brainstem circuitry. A basis exists for attributing the minor proconvulsive effects reported for aspartame in a few experiments to uncontrolled variables.

7.7 Aspartame and absence seizures

Studies targeting absence seizures in mammalian models of human epilepsy have not been undertaken. Pentylenetetrazol-induced convulsions have been historically employed as a a step in identifying new drugs for the treatment of this disorder (Loscher and Schmidt, 1988). Proconvulsant effects of aspartame were not established in the pentylenetetrazol model. This lack of seizure facilitation may be interpreted as an indication of the safety of aspartame in cases of absence epilepsy. Accordingly, Loscher and Schmidt proposed as recently as 1988 that "generalized clonus with loss of righting reflexes induced by" pentylenetetrazol might be a relatively reliable endpoint in gross screening tests. However, the effects of aspartame were determined in protocols using pentylenetetrazol-induced generalized clonus with loss of posture in rodents. Without a doubt, these are convulsive seizures, whereas absence seizures in humans are non-

convulsive. Seemingly, this difference renders the pentylenetetrazol test less than dependable in studies attempting to model absence seizures. In keeping with this idea, Loscher and colleagues (1991b) now believe that this test yields results that are subject to misleading interpretations relative to absence seizures. Two pharmacologically predictive models of absence epilepsy have now been developed. These include the genetic absence epilepsy rat (Vergnes and colleagues, 1990) and the gamma-hydroxy-butyrate treated rat (Snead, 1988).

Three clinical studies have been completed which include subjects with absence seizures. The two studies comparing aspartame with inert placebo, both being given orally in the form of capsules, have found that aspartame had no effect on absence seizures. The other, comparing aspartame sweetened fluid with sucrose sweetened fluid, reports that the mean number of seconds per hour spent in spike-wave discharges was greater after aspartame compared with sucrose.

8 Conclusions

Convulsive seizures are not caused by orally administered aspartame in mammals including baboons and humans. Moreover, aspartame does not facilitate convulsions produced by seizure inducing stimuli. Proconvulsive effects are absent in humans and other mammals with epilepsy and in those without epilepsy. They are missing both in forebrain and brainstem seizures. Lack of convulsive liability is apparent even when the doses of aspartame are extreme compared to those consumed in the human diet. Studies of aspartame in absence seizures are not as complete as those in convulsive seizures, but available evidence in humans does not document an association between absence seizure incidence and aspartame usage.

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