

Research Reports

Lack of effect of aspartame or of L-phenylalanine on photically induced myoclonus in the baboon, *Papio papio*

B.S. Meldrum^a, N. Nanji^a and R.G. Cornell^b

^aDepartment of Neurology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF (U.K.), and

^bDepartment of Biostatistics, University of Michigan, Ann Arbor, MI 48109-2029 (U.S.A.)

(Received 14 July 1988; accepted 5 October 1988)

Key words: Epilepsy; Baboons; L-Phenylalanine; Aspartame; Myoclonus

The effects of large doses of L-phenylalanine and of aspartame on seizure susceptibility and severity have been assessed in baboons *Papio papio* from Senegal which show photosensitive epileptic responses similar to primary generalised epilepsy in man. L-Phenylalanine, 50, 150 or 450 mg/kg, or aspartame, 300 or 1000 mg/kg, were administered orally. Peak plasma L-phenylalanine concentrations of approximately 2000 μ moles/l occurred 1-4 h after the highest dose of L-phenylalanine or aspartame. The plasma L-phenylalanine to large neutral amino acid ratio increased approximately 30-fold at this time. Compared with water administration there were no changes in epileptic responses 1-5 h after either treatment. In this primate model of epilepsy acute increases in plasma phenylalanine concentration are neither pro- nor anticonvulsant.

INTRODUCTION

It has been suggested that the consumption of high doses of the sweetener aspartame (L-aspartyl-L-phenylalanine methyl ester) can precipitate seizures in susceptible subjects²⁹. To date, however, no documented cases have been published. Nevertheless the suggestion requires investigation because of the potential importance of the problem in public health terms and because there are conflicting data concerning a putative proconvulsant effect of aspartame or of its hydrolysis product L-phenylalanine in rodent models of epilepsy. Chronic administration of L-phenylalanine (Phe)

reportedly enhances reflexly induced seizure responses in mouse strains which are susceptible to audiogenic convulsions²⁶. Acutely aspartame, 1000 mg/kg, or L-phenylalanine, 560 mg/kg, have been reported to enhance the convulsant effect of pentylenetetrazol or flurothyl in normal mice²². A lack of effect of aspartame, 1000-2500 mg/kg, has however, been reported in kindled, electroshock and sound-induced seizures in rats^{12,13,25} and in pentylenetetrazol and electroshock seizures in mice^{11,21}.

Two effects of phenylalanine could be relevant to seizure susceptibility. Firstly, Phe can compete with tyrosine (Tyr) for uptake into the brain (by the large neutral amino acid carrier)³. Secondly, Phe can inhibit the enzyme tyrosine hydroxylase, which catalyses the rate-limiting step in the synthesis of norepinephrine¹⁰. Both these effects

Correspondence to: Prof. B.S. Meldrum, Institute of Psychiatry, Department of Neurology, De Crespigny Park, Denmark Hill, London SE5 8AF, U.K.

would be expected to decrease the synthesis of catecholamines in the brain.

Direct evidence for an effect of Phe on seizure threshold in primates is so far lacking. We have, therefore, studied the effect of the acute oral administration of L-phenylalanine or of aspartame on photically induced myoclonic responses and on plasma concentrations of large neutral amino acids in *Papio papio* from the Casamance region of Senegal²⁰. This model is highly susceptible to manipulation of brain monoamines. Dopamine agonists in low doses (e.g., apomorphine) have a protective effect^{2,19}. The myoclonic responses to photic stimulation are also blocked by the intraventricular administration of norepinephrine¹. Chronic reserpine administration (leading to non-selective depletion of cerebral monoamines) facilitates myoclonic responses in animals initially showing low responsivity¹. Photically induced myoclonus in this primate model corresponds closely in its electrophysiology and pharmacological responsiveness to primary generalised epilepsy in man. Thus, the photosensitive baboon would appear to be an appropriate animal model for testing the effect of aspartame or L-phenylalanine on seizure threshold.

MATERIALS AND METHODS

Adolescent baboons from the Casamance region of Senegal were individually caged and maintained under constant environmental conditions. They were seated in primate chairs and tested for photically induced myoclonic responses as described by Meldrum et al.¹⁷. Four highly photosensitive adolescent baboons (2 males, 2 females, weights 6–8 kg) were selected for the Phe study. Each animal received (in random order, at weekly intervals) 0, 50, 150 or 450 mg of phenylalanine/kg by gavage. Myoclonic responses to a standardised 5 min period of photic stimulation, prior to and at hourly intervals after drug administration, were scored according to Meldrum et al.¹⁷. Animals were closely observed throughout the 6–7 h period in the chair. On one-third of the days on which animals were tested, blood samples (from a femoral venous catheter) were taken into heparinised tubes prior to and at 0.5, 1, 2, and 4 h after Phe ad-

TABLE I

Study of the acute effect of L-phenylalanine on myoclonic responses in photosensitive baboons, Papio papio

Myoclonic responses graded 0–4 as in Meldrum et al.¹⁷. Each value represents the mean score in 4 baboons tested at the time indicated prior to or after an administration of L-phenylalanine or vehicle.

Oral dose (mg/kg)	Time (min)	Myoclonic response					
		–60	45	105	165	225	285
0		3.75	3.0	2.5	2.25	2.0	2.25
50		3.75	2.75	2.00	2.25	2.0	2.25
150		3.75	2.75	2.5	1.75	2.0	2.0
450		3.75	3.5	2.5	2.25	2.25	2.25

ministration. Plasma amino acid concentrations were determined by ion exchange chromatography (Hazelton Laboratories, Madison, WI). De-proteinised samples were diluted with an internal standard and analysed on a Beckman 7300 amino acid analyzer with post-column derivatization. The ratios of the phenylalanine and tyrosine concentration to the sum of the other large neutral amino acids, LNAA (considering valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and tryptophan as the LNAA), were calculated for each time point.

For the aspartame study 4 further baboons were selected (3 females, weights 4–5 kg, 1 male, weight 5 kg) with high (n = 1) or intermediate (n = 3) photosensitivity (to facilitate the demonstration of minor proconvulsant effects). Each animal received, orally, tap water, aspartame 300 mg/kg, or aspartame 1000 mg/kg on 3 occasions (random order replicated Latin squares design⁷), at weekly intervals over a 3 month period. In each baboon venous blood samples were taken for plasma amino acid analysis (as in the Phe study) on 1 out of the 3 occasions with a given treatment. On every occasion myoclonic responses were assessed prior to, and at hourly intervals after, aspartame administration.

Statistical analysis

The decreases in myoclonic scores from baseline were averaged over time for each series of observations on a baboon. These average decreases were ranked within blocks, with mid-ranks calcu-

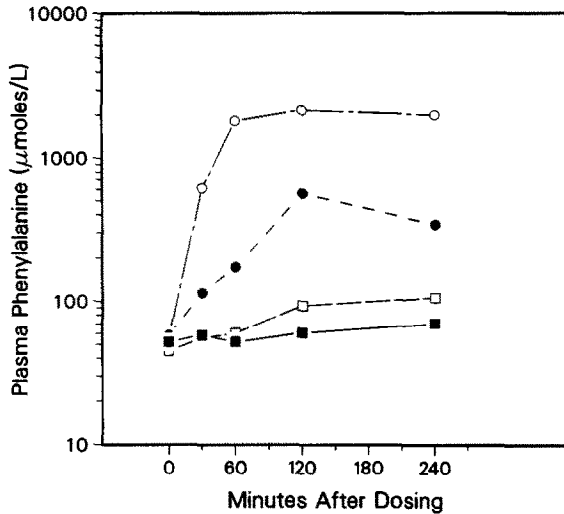


Fig. 1. Changes in plasma phenylalanine concentration after oral dosing of Phe (■, 0; □, 50; ●, 150; and ○, 450 mg/kg) in *Papio papio*. Each value represents the mean of measurements in 2 baboons.

lated for ties. Friedman's⁹ procedure was used to test the null hypothesis of no dose effect against a non-specific alternative hypothesis. Page's⁹ test, which is more sensitive against the alternative hypothesis of a monotonic trend in responses for increasing doses, was also calculated when a monotonic trend in average ranks was observed. For the L-phenylalanine experiment, the series of observations were blocked by baboon for purposes of comparing responses to the 4 doses. For the aspartame experiment, each of the 3 doses was used once each day, so that the doses were compared within days in the computation of ranks.

RESULTS

L-Phenylalanine

Similar decreases in the severity of the myoclonic response to photic stimulation were observed over time after each treatment (Table I). Ranking of the decreases in seizure score showed that there was no significant difference in dosage effects (Friedman's test, empirical significance level $P = 0.34$).

Peak plasma Phe concentrations of 60, 93, 561 and 2155 $\mu\text{moles/l}$ were observed approximately 120 min after administration of Phe, 0, 50, 150 and 450 mg/kg, respectively (see Fig. 1). In contrast,

TABLE II

The acute effect of aspartame on myoclonic responses in photosensitive baboons, Papio papio

Myoclonic responses graded 0–4 as in Meldrum et al.¹⁷. Each value represents the mean score for 4 baboons on 3 occasions at the times indicated prior to and after oral administration of aspartame or tap water.

Oral dose (mg/kg)	Time (min)	Myoclonic response					
		-60	45	105	165	225	285
0		1.83	1.16	1.0	0.58	0.66	0.83
300		1.41	1.25	0.83	0.75	0.75	0.75
1000		1.5	0.66	0.66	0.66	0.58	0.58

plasma tyrosine levels did not exceed 78 $\mu\text{moles/l}$ in any baboon throughout the 4 h sampling period (Fig. 2). Calculation of the plasma Phe:large neutral amino acid ratio (a predictor of the competitive uptake of phenylalanine into brain) revealed a dose-related 2-, 11- and 34-fold increase compared to control (Fig. 3). Thus even at extremely high concentrations of plasma Phe, neither pro- nor anticonvulsant effects were observed in baboons.

Aspartame

In all 4 baboons the scores for the myoclonic responses were decreased after administration of tap water or aspartame (see Table II). Statistical analysis revealed no effect of treatment (Friedman's test, $0.7 < P < 0.8$; Page's test, $P = 0.48$).

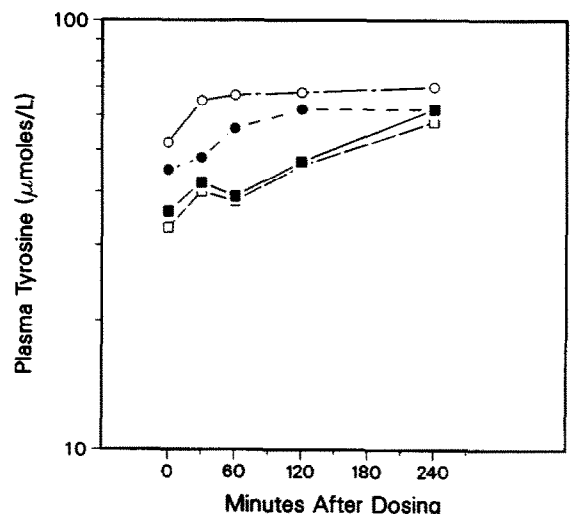


Fig. 2. Changes in plasma tyrosine concentration after oral dosing of Phe (■, 0; □, 50; ●, 150; and ○, 450 mg/kg). Values are the means of measurements in 2 baboons.

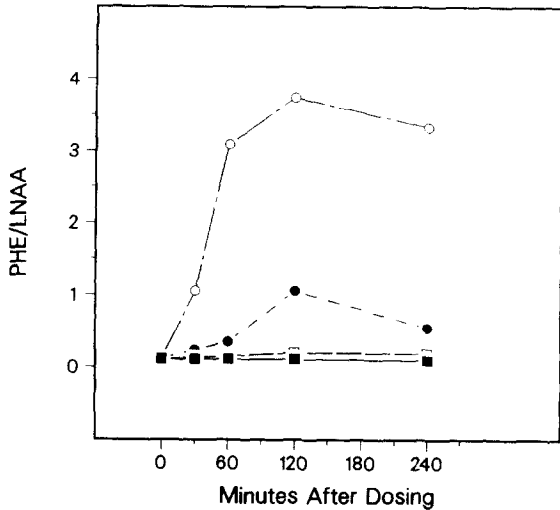


Fig. 3. Changes in the ratio of Phe to the sum of the large neutral amino acids in the plasma of baboons after Phe ■, 0; □, 50; ●, 150; and ○, 450 mg/kg.

Plasma amino acid measurements showed that control (tap water) animals had Phe levels around 50–60 μ moles/l (see Fig. 4). Peak Phe concentration after an aspartame dose of 1000 mg/kg (Fig. 4) was close to that observed after administration of Phe in a dose of 450 mg/kg (Fig. 1). In each of these cases, Phe concentrations were close to 2000 μ moles/l. However, the peak after Phe occurred at

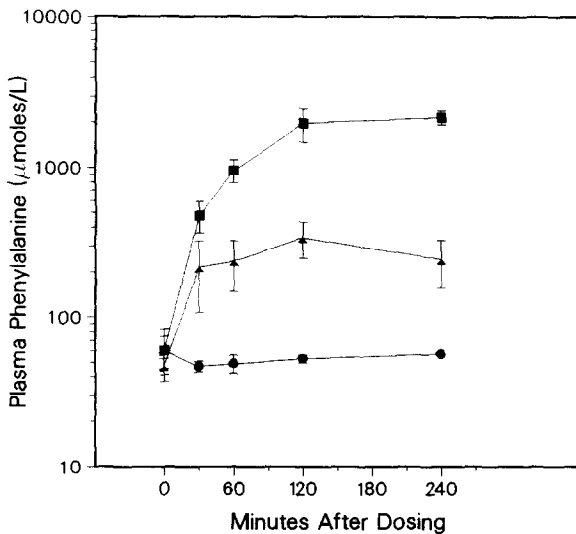


Fig. 4. Changes in plasma phenylalanine concentration after oral dosing of aspartame (●, 0; ▲, 300; and ■, 1000 mg/kg) in *Papio papio*. Each value represents the mean of measurements in 4 baboons. Error bars represent the standard deviation.

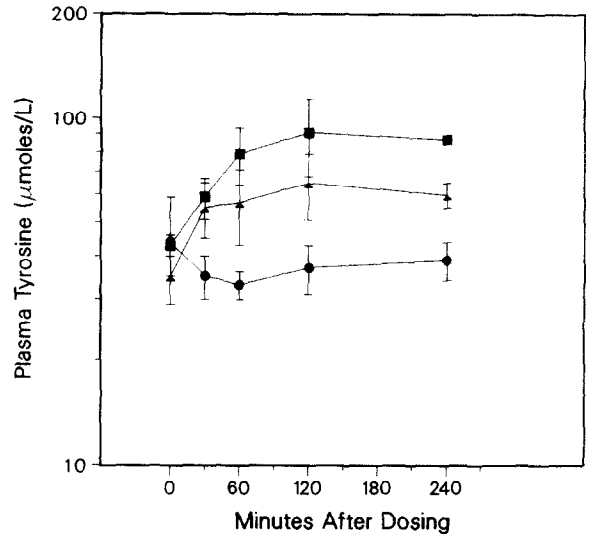


Fig. 5. Ratio of Phe to the sum of the large neutral amino acids in plasma after aspartame ●, 0; ▲, 300; and ■, 1000 mg/kg orally in 4 baboons. Error bars represent the standard deviation.

about 60 min, whereas the peak after aspartame occurred at approximately 120 min. The effect of aspartame 300 mg/kg on plasma Phe content was similar to that of Phe 150 mg/kg, but with a lower peak value. Control plasma tyrosine values were in the range 33–44 μ moles/l (Fig. 5). Plasma tyrosine values were between 79 and 91 μ moles/l, 1–4 h after an aspartame dose of 1000 mg/kg. In the latter aspartame experiments the mean baseline

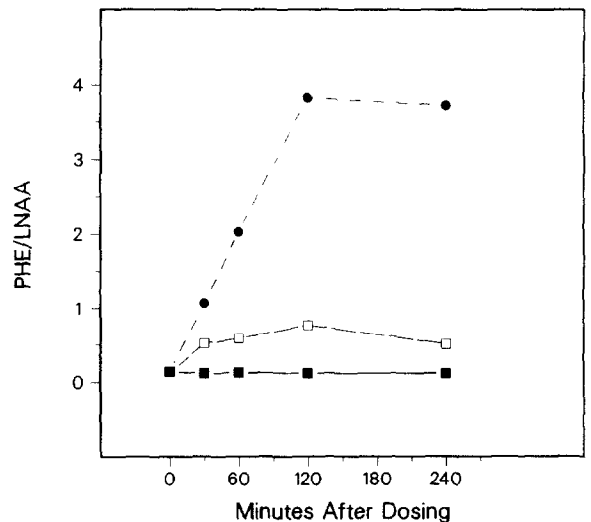


Fig. 6. Ratio of Phe to the sum of the large neutral amino acids in plasma after aspartame ■, 0; □, 300; and ●, 1000 mg/kg orally in 4 baboons.

value of the Phe/LNAA was 0.14 (see Fig. 6). This rose to 3.83 at 2 h after aspartame 1000 mg/kg (see Fig. 6). The Tyr/LNAA fell from a pretreatment value of 0.095–0.033 at 4 h (data curves not shown). The Phe/Tyr rose from 1.39 (pretreatment) to 24.7 at 4 h (curves not shown).

DISCUSSION

This study shows no effect (either proconvulsant or anticonvulsant) of high acute oral doses of L-phenylalanine or aspartame in baboons with photosensitive epilepsy. This contrasts with the known sensitivity of this animal model to manipulation of monoaminergic systems, including noradrenergic, dopaminergic and serotonergic activity. It also contrasts with some, but not all, published reports concerning sound-induced and chemically induced seizures in rodents. The proconvulsant action reported in DBA/2 mice at 22 days of age followed the administration of Phe as 2% of the diet to the mother and weanling²⁶. In mice, an acute oral dose of aspartame 1000–2000 mg/kg or an equimolar dose of phenylalanine reportedly has a proconvulsant action, when administered 1 h prior to pentylenetetrazol or flurothyl²². Jobe et al.¹¹, however, found no potentiation of the convulsant effect of pentylenetetrazol in fasted or fed mice 1 h after aspartame 1500–2500 mg/kg. Nevins et al.²¹ similarly found no effect of aspartame on electroshock or pentylenetetrazol seizure thresholds in mice. In rats, Garrattini et al.⁸ found a weak potentiation of pentylenetetrazol's convulsant action 60 min after aspartame, 1000 mg/kg orally. However, threshold currents for electroshock were not altered by aspartame administration. A lack of effect of aspartame in rats has also been reported for the rate of kindling²⁵, electroshock seizure threshold²⁵, supramaximal electroshock seizure severity¹³ and sound-induced seizures¹².

The peripheral metabolism of the amino acids that are precursors for monoamine neurotransmitters is quantitatively different in rodents compared with primates. Central nervous system metabolism may also be different. In particular, an oral dose of phenylalanine in rats produces a very marked increase in plasma tyrosine concentration, probably because of the high activity of phenylala-

nine hydroxylase in rat liver³⁰. In man, oral doses of phenylalanine or aspartame produce changes in plasma amino acid concentrations essentially equivalent to those we have found in baboons. Thus, normal adults receiving aspartame 100 or 200 mg/kg show peak plasma concentrations of phenylalanine of 202 or 487 μ moles/l after 0.75–1.5 h^{23,24}. Tyrosine concentrations show a small but more prolonged elevation (from control values around 50 μ moles/l to peak values of 80–90 μ moles/l). For rodents, some data are available about changes in brain content of monoamines (and their extracellular concentration) following administration of high doses of phenylalanine or aspartame, but the details and interpretation remain obscure. In rats aspartame, 200 mg/kg, produced a marked elevation of brain tyrosine and phenylalanine content, but had only small and inconsistent effects on norepinephrine and did not alter brain tryptophan or serotonin content^{31,32}. Garrattini et al.⁸ found no changes in rat brain content of dopamine, 5-hydroxytryptamine or norepinephrine (or their metabolites) after aspartame 250 mg/kg orally. In mice aspartame, 130 or 650 mg/kg orally, increased norepinephrine content by 50% in the hypothalamus and by smaller amounts elsewhere in the brain⁴. The content of dopamine and of various catecholamine metabolites was also increased. These findings do not support the assumption that catecholamine synthesis would be impaired by a high plasma phenylalanine concentration. Preliminary studies using intracerebral dialysis in rats receiving phenylalanine intraperitoneally indicate an increased release of dopamine after Phe, 200 mg/kg, but a decreased release after Phe, 1000 mg/kg⁵. No data are available about effects of Phe or aspartame on cerebral monoamine content or release in primates.

Reports of seizures in man following aspartame consumption principally concern the acute consumption of large volumes of beverages (often with low sodium content)²⁹. There is a high probability that such seizures are attributable to rapid rehydration and hyponatraemia, a situation long known to precipitate seizures in patients with epilepsy or a reduced seizure threshold associated with cerebral pathology^{14,28}. Controlled laboratory studies designed to differentiate the effects of

water load and aspartame on seizure threshold in man have not been reported. The possible effect of chronic administration of Phe or aspartame has not been addressed in this study. The absence of epidemiological data showing an increase in the incidence of new cases of epilepsy subsequent to the introduction of aspartame as a sweetener suggests that this is either not a widespread phenomenon or that it is a non-existent event. It remains possible that neurochemically specific syndromes of epilepsy might be affected by alterations in plasma Phe. The two human epileptic syndromes that are modified by monoaminergic manipulation (i.e., photo-

myoclonic responses blocked by dopamine agonists and some types of post-hypoxic myoclonus that respond to tryptophan or 5-hydroxytryptophan) are accurately modelled (in pharmacological terms) by photosensitive responses in the baboon^{2,18,27}. We conclude that there is no evidence for a proconvulsant action of very high acute doses of Phe or aspartame in primates.

ACKNOWLEDGEMENTS

We thank The Wellcome Trust and The Nutra-Sweet Company for financial support.

REFERENCES

- 1 Altschuler, H.L., Killam, E.K. and Killam, K.F., Biogenic amines and the photomyoclonic syndrome in the baboon, *Papio papio*, *J. Pharmacol. Exp. Ther.*, 196 (1976) 156–166.
- 2 Anlezark, G., Marrosu, F. and Meldrum, B., Dopamine agonists in reflex epilepsy. In: P.L. Morselli, K.G. Lloyd, W. Löscher, B. Meldrum and E.H. Reynolds (Eds.), *Neurotransmitters, Seizures and Epilepsy*, Raven Press, New York, 1981, pp. 251–262.
- 3 Chirigos, M., Greengard, P. and Udenfriend, S., Uptake of tyrosine by rat brain in vivo, *J. Biol. Chem.*, 235 (1960) 2075–2079.
- 4 Coulombe, R.A. and Sharma, R.P., Neurobiochemical alterations induced by the artificial sweetener aspartame (NutraSweet), *Toxicol. Appl. Pharmacol.*, 83 (1986) 79–85.
- 5 During, M.J., Acworth, I.N. and Wurtman, R.J., Phenylalanine administration influences dopamine release in the rat's corpus striatum, *Neurosci. Lett.*, 93 (1988) 91–95.
- 6 Fernstrom, J.D. and Wurtman, R.J., Brain-serotonin content: physiological regulation by plasma neutral amino acids, *Science*, 178 (1972) 414–416.
- 7 Fleiss, J.L., *Design and Analysis of Clinical Experiments*, Wiley, New York, 1986, pp. 249–255.
- 8 Garattini, S., Caccia, S., Romano, M., Diomedea, L., Guiso, G., Vezzani, A. and Salmons, M., Studies on the susceptibility to convulsions in animals receiving abuse doses of aspartame. In: R.J. Wurtman and E. Ritter-Walker (Eds.), *Dietary Phenylalanine and Brain Function*, Center for Brain Sciences, Cambridge, MA, 1987, pp. 129–142.
- 9 Hollander, M. and Wolfe, D.A., *Nonparametric Statistical Methods*, Wiley, New York, 1973, pp. 138–150.
- 10 Ikeda, M., Levitt, M. and Udenfriend, S., Phenylalanine as a substrate and inhibitor of tyrosine hydroxylase, *Arch. Biochem. Biophys.*, 120 (1967) 420–427.
- 11 Jobe, P.C., Bettendorf, A.F., Lasley, S.M. and Dailey, J.W., Effects of aspartame on pentylenetetrazol (PTZ)-induced convulsions in CD-1 mice, *Toxicologist*, 8 (1988) 85.
- 12 Jobe, P.C., Lasley, S.M. and Dailey, J.W., Monoamines and seizures in genetically epilepsy-prone rats: inferences from precursor availability. In: G. Huether (Ed.), *Amino Acid Availability and Brain Function in Health and Disease*, Springer, Berlin, 1988, pp. 207–214.
- 13 Jobe, P.C., Lasley, S.M., Bettendorf, A.F., Frasca, J.J. and Dailey, J.W., Studies of aspartame on supramaximal electroshock seizures in epileptic and non-epileptic rats, *FASEB J.*, 2 (1988) A1067.
- 14 McQuarrie, I. and Peeler, D.B., The effects of sustained pituitary antidiuresis and forced water drinking in epileptic children. A diagnostic and etiologic study, *J. Clin. Invest.*, 10 (1931) 915–940.
- 15 Meldrum, B.S., Photosensitive epilepsy in *Papio papio* as a model for drug studies, *Electroenceph. Clin. Neurophysiol.*, Suppl. 34 (1978) 317–322.
- 16 Meldrum, B.S., The effect of psychotropic drugs on the seizure threshold; animal studies. In: M.R. Trimble (Ed.), *The Psychopharmacology of Epilepsy*, Wiley, Chichester, 1985, pp. 107–117.
- 17 Meldrum, B.S., Balzano, E., Gadea, M. and Naquet, R., Photic and drug-induced epilepsy in the baboon (*Papio papio*): the effects of isoniazid, thiosemicarbazide, pyridoxine and amino-oxyacetic acid, *Electroenceph. Clin. Neurophysiol.*, 29 (1970) 333–347.
- 18 Meldrum, B.S., Balzano, E., Wada, J.A. and Vuillon-Cacciuttolo, G., Effects of L-tryptophan, L-3,4-dihydroxyphenylalanine and tranlycypromine on the electroencephalogram and on photically induced epilepsy in the baboon, *Papio papio*, *Physiol. Behav.*, 9 (1972) 615–621.
- 19 Meldrum, B.S., Anlezark, G.F. and Trimble, M., Drugs modifying dopaminergic activity and behaviour, the EEG and epilepsy in *Papio papio*, *Eur. J. Pharmacol.*, 32 (1975) 203–215.
- 20 Naquet, R. and Meldrum, B.S., Photogenic seizures in baboons. In: D.P. Purpura, D.B. Tower, D.M. Woodbury

- and R.D. Walter (Eds.), *Experimental Models of Epilepsy*, Raven Press, New York, 1972, pp. 373–406.
- 21 Nevins, M.E., Arnolde, S.M. and Haigler, H.J., Aspartame; lack of effect on convulsant thresholds in mice, *Fed. Proc.*, 45 (1986) 1629–1639.
 - 22 Pinto, J.M.B. and Maher, T.J., Administration of aspartame potentiates pentylenetetrazole- and flurothyl-induced seizures in mice, *Neuropharmacology*, 27 (1988) 51–55.
 - 23 Stegink, L.D., Aspartame metabolism in humans: acute dosing studies. In: L.D. Stegink and L.J. Filer (Eds.), *Aspartame. Physiology and Biochemistry*, Marcel Dekker, New York, 1984, pp. 509–553.
 - 24 Stegink, L.D., Filer, L.J. and Baker, G.L., Plasma and erythrocyte concentrations of free amino acids in adult humans after abuse doses of aspartame, *J. Toxicol. Environ. Hlth*, 7 (1981) 291–305.
 - 25 Thai, L., Tilson, H.A., Zhao, D., Sbotka, T. and Hong, J.S., Lack of effect of aspartame in kindling, electroshock (ECS) and metrazol-induced seizures in rats, *Neurosci. Abst.*, 14 (1988) 866.
 - 26 Truscott, T.C., Effects of phenylalanine and 5-hydroxytryptophan on seizure severity in mice, *Pharmacol. Biochem. Behav.*, 3 (1975) 939–941.
 - 27 Wada, J.A., Balzamo, E., Meldrum, B.S. and Naquet, R., Behavioural and electrographic effects of L-5-hydroxytryptophan and D,L-parachlorophenylalanine on epileptic Senegalese baboon, *Papio papio*, *Electroenceph. Clin. Neurophysiol.*, 33 (1972) 520–526.
 - 28 Worthley, L.I.G. and Thomas, P.D., Treatment of hyponatraemic seizures with intravenous 29.2% saline, *Br. Med. J.*, 292 (1986) 168–170.
 - 29 Wurtman, R.J., Aspartame; possible effect on seizure susceptibility, *Lancet*, ii (1985) 1060.
 - 30 Wurtman, R.J. and Maher, T.J., Effects of oral aspartame on plasma phenylalanine in humans and experimental rodents, *J. Neural Transm.*, 70 (1987) 169–173.
 - 31 Yokogoshi, H., Roberts, C.H., Cabellero, B. and Wurtman, R.J., Effects of aspartame and glucose administration on brain and plasma levels of large neutral amino acids and brain 5-hydroxyindoles, *Am. J. Clin. Nutr.*, 40 (1984) 1–7.
 - 32 Yokogoshi, H. and Wurtman, R.J., Acute effects of oral or parenteral aspartame on catecholamine metabolism in various regions of rat brain, *J. Nutr.*, 116 (1986) 356–364.