British Journal of Nutrition (2001), **86**, 151–155
DOI: 10.1079/BJN2001360

Short communication

Effects of dietary sucrose on hippocampal serotonin release: a microdialysis study in the freely-moving rat

Ilse Smolders[1], Jan Van Loo[2], Sophie Sarre[1], Guy Ebinger[3] and Yvette Michotte[1]*
[1]*Department of Pharmaceutical Chemistry and Drug Analysis, Pharmaceutical Institute, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium*
[2]*ORAFTI, Aandorenstraat 1, 3300 Tienen, Belgium*
[3]*Department of Neurology, University Hospital, Laarbeeklaan 101, 1090 Brussels, Belgium*

(Received 27 March 2000 – Revised 2 January 2001 – Accepted 29 January 2001)

The effects of dietary supplementation with either sucrose or starch (50 g/kg regular food for 2 weeks) on central 5-hydroxytryptamine (5HT; serotonin) release were investigated in freely-moving rats. It has been suggested that the amount of transmitter that serotoninergic neurons release might be altered by food intake. We monitored the effects of sucrose and starch on concentrations of extracellular 5HT, its metabolite 5-hydroxyindoleacetic acid (5HIAA), γ-aminobutyric acid (GABA) and dopamine in the hippocampus, using *in vivo* microdialysis. The major finding was that baseline levels of extracellular hippocampal 5HT in rats with *ad libitum* access to food supplemented with sucrose were significantly higher compared with the starch control group. We then verified that sucrose supplementation affected the potency of S(+)fenfluramine to increase hippocampal 5HT levels. In both groups of rats, acute intraperitoneal injection (1 mg/kg) of this anorectic drug induced a response curve of the extracellular hippocampal 5HT levels, with a shape that corresponded with earlier data for different brain areas often using up to 10-fold higher doses of S(+)fenfluramine. Nevertheless, we showed that throughout the experiment the absolute values of the sucrose response curve remained higher than in the starch group. On the other hand, S(+)fenfluramine exerted longer lasting effects in the starch group, as compared with the sucrose group. Significant decreases in levels of extracellular hippocampal 5HIAA levels following S(+)fenfluramine administration were simultaneously observed. A practical implication of the present findings is that dietary sucrose may bias the results of studies investigating brain serotoninergic mechanisms and the effects of (anorectic) drugs interacting with 5HT systems in the hippocampus.

Sucrose: Serotonin: Microdialysis

Evidence has accumulated for a key role of central serotoninergic mechanisms in appetite regulation, carbohydrate craving and the control of mood (Wurtman & Wurtman, 1995; Blundell & Halford, 1998). Accordingly, 5-hydroxytryptamine (5HT; serotonin) release is tightly controlled by food intake and meal composition (Rouch *et al.* 1999). The rate-limiting step in 5HT synthesis is hydroxylation of tryptophan, implying that tryptophan alterations can modify 5HT synthesis rate and hence brain 5HT concentrations (Leathwood, 1987). The hypothesis that 5HT release is affected by a sudden elevation of tryptophan availability was confirmed by several microdialysis approaches performed in different rat brain regions (Carboni *et al.* 1989; Schwartz *et al.* 1990; Sharp *et al.* 1992; Sarre *et al.* 1997). Tryptophan is taken up into the brain by an active transport system for

Abbreviations: GABA, γ-aminobutyric acid; 5HIAA, 5-hydroxyindoleacetic acid; 5HT, 5-hydroxytryptamine (serotonin).

* **Corresponding author:** Professor Yvette Michotte, fax +32 2 477 41 13, email ymichot@minf.vub.ac.be

large neutral amino acids. This implies that brain tryptophan levels depend not only on plasma tryptophan concentrations, but also on the levels of all other large neutral amino acids (Young, 1991; Stancampiano *et al.* 1997). A carbohydrate-rich meal does not alter the amount of plasma tryptophan *per se* but the carbohydrates cause acute insulin release, which results in uptake of glucose and the branched-chain amino acids, leucine, isoleucine and valine, into muscles. As a consequence, the branched-chain amino acid plasma levels decline, the value of the plasma tryptophan:large neutral amino acid ratio increases, and brain tryptophan and 5HT levels rise (Leathwood, 1987; Young, 1991). Besides these well-described acute effects, it has also been shown that chronic administration of certain carbohydrates can significantly alter blood insulin concentrations. Plasma insulin levels, measured after 5 h food deprivation, were approximately doubled in rats fed for 12 d with a diet in which all vegetable starch (600 g/kg) was replaced with either glucose or sucrose (Reaven & Ho, 1991). Moreover, in another experiment on the chronic effects of the source of the dietary carbohydrate (i.e. starch, fructose, glucose or sucrose) on blood insulin levels, Thibault (1994) demonstrated that the highest insulin concentrations were found in sucrose-fed rats, while the lowest levels were obtained in starch-fed animals. In the latter experiment, rats were given access to their food mixture *ad libitum* for 8 h/d and insulin content was determined immediately after the feeding period. Lastly, Young & Landsberg (1977) showed that a few days of sucrose feeding significantly stimulated sympathetic activity, which in turn can raise brain tryptophan levels (Eriksson & Carlsson, 1982; Eriksson *et al.* 1984; Edwards *et al.* 1989).

On the basis of these findings described earlier, we investigated the effects of chronic dietary supplementation with sucrose (50 g/kg diet for 2 weeks) *v.* starch as placebo on basal and S(+)fenfluramine-induced hippocampal 5HT release in conscious rats, using *in vivo* microdialysis. These sugars are often part of the standard diet of experimental animals used in studies focusing on serotoninergic mechanisms. We simultaneously monitored the effects of sucrose and S(+)fenfluramine on the extracellular hippocampal concentrations of 5-hydroxyindoleacetic acid (5HIAA), the direct metabolite of 5HT. The anorectic drug S(+)fenfluramine increases extracellular 5HT by rapidly inhibiting 5HT re-uptake, but also, at high doses, by releasing 5HT (Garattini *et al.* 1992; van Kempen, 1994; Caccia *et al.* 1997). In the present study, S(+)fenfluramine (intraperitoneal injection, 1 mg/kg) was administered acutely on the day of the microdialysis experiment. We further studied possible effects of dietary sucrose supplementation on the basal extracellular levels of dopamine and γ-aminobutyric acid (GABA) in the hippocampus.

Materials and methods

Experimental protocol

Sucrose (α,D-glucopyranosyl-β,D-fructofuranoside) and starch (amylose–amylopectin glucan chains) were purchased from Merck (Darmstadt, Germany). Regular food (Rats & Souris Entretien "A 04") in the form of powder was obtained from Animalabo (Brussels, Belgium).

Male albino Wistar rats (start body weight 200 g) were divided into a sucrose group (*n* 7) and a starch control group (*n* 7). For 2 weeks, animals from the sucrose group were fed a mixture of regular food with added sucrose (50 g/kg diet), while the starch group were fed a mixture of regular food supplemented with starch (50 g/kg diet). These food mixtures were designed to be isoenergetic.

The protocol is in accordance with National Rules on Animal Experiments and was approved by the Ethics Committee on Animal Experiments of the Faculty of Medicine and Pharmacy of the Free University of Brussels, Belgium. Body weight was determined on the day before the microdialysis experiment. The animal was anaesthetised with ketamine-diazepam (25:5, w/w; 30 mg/kg) and mounted on a stereotaxic frame. An intracranial guide cannula (CMA/Microdialysis, Stockholm, Sweden) was implanted into the dorsal hippocampus, exactly 3 mm above the area to be dialysed. Coordinates towards bregma were L +4·6, A −5·6 and V +4·6 (Paxinos & Watson, 1986). Immediately following surgery, rats received an injection of ketoprofen (4 mg/kg) to provide post-operative analgesia and the guide cannula obturators were replaced by CMA10/Microdialysis probes (Stockholm, Sweden, membrane length 3 mm). Probes were continuously perfused with Ringer's solution at a flow rate of 1 μl/min. The aqueous Ringer's solution contained 147 mM-NaCl, 2·3 mM-$CaCl_2$ and 4 mM-KCl. The rats were allowed to recover from surgery overnight and had free access to water and their food mixture.

On the day of the microdialysis experiment, dialysates were collected every 20 min from the freely-moving rats, yielding 20 μl samples. Twelve hippocampal dialysates were sampled under baseline conditions in each animal: four for on-line 5HT–5HIAA analysis, four for GABA analysis, and four for dopamine analysis (crossover design). The collection vials for dopamine measurements contained 5 μl antioxidant mixture, consisting of 0·02 mM-HCl, 2 g sodium metabisulphite/l and 0·2 g Na_2EDTA/l. The animals received an intraperitoneal injection of S(+)fenfluramine (1 mg/kg in physiological saline) at the beginning of collection period 13. Dialysates were collected for a further period of 2 h (collection periods 14 to 19) and analysed on-line for 5HT and 5HIAA. At the end of the experiments, rats were killed with an overdose of pentobarbital.

Liquid chromatography sample analysis

Concentrations of 5HT and 5HIAA in the dialysates were determined by reversed-phase ion-pair microbore liquid chromatography with dual electrochemical detection (Sarre *et al.* 1992). Measurements of GABA, after pre-column derivatisation (Smolders *et al.* 1995), and dopamine (Smolders *et al.* 1996) in the dialysates were carried out by reversed-phase ion-pair microbore liquid chromatography with electrochemical detection.

Statistical analysis

The results of each group were expressed as mean extracellular neurotransmitter or metabolite concentrations with standard error of the mean, in μM for GABA and 5HIAA and in nM for 5HT and dopamine. The baseline values were calculated as the mean of the stable transmitter or 5HIAA dialysate concentrations obtained before S(+)fenfluramine administration. Dialysate levels were not corrected for recovery across the dialysis membrane. Baseline levels of the different neurotransmitters and 5HIAA in both groups were compared using Mann-Whitney's test (two-tailed, $\alpha = 0{\cdot}05$). Statistical analysis of changes in the concentrations of 5HT or 5HIAA with time due to drug administration was performed by one-way ANOVA for repeated measures and Fisher's protected least significant difference *post hoc* test (two-tailed, $\alpha = 0{\cdot}05$). The significance of differences between peak dialysate levels was tested by Mann-Whitney's test (two-tailed, $\alpha = 0{\cdot}05$).

Results and discussion

Rat body weights

The body weights of the animals of the sucrose group were 263·8 (SEM 4·5) g. These body weights did not differ significantly from those recorded for the starch group, i.e. 267·8 (SEM 17·0) g (Mann-Whitney's test, $\alpha = 0{\cdot}05$). This might be taken to confirm that the food mixtures were indeed isoenergetic.

Baseline hippocampal dialysate concentrations

The results shown in Table 1 are the mean basal extracellular concentrations of the different neurotransmitters and 5HIAA in rat hippocampus. The baseline extracellular 5HT concentrations obtained in the sucrose group (2·332 (SEM 0·766) nM) were significantly ($P = 0{\cdot}024$) higher compared with the 5HT levels obtained in the starch group (0·396 (SEM 0·097) nM). The baseline extracellular 5HT levels in the starch group were comparable with the baseline hippocampal 5HT levels of a pooled control population from former experiments, i.e. 0·386 (SEM 0·060) nM (n 25). We observed no statistical difference between basal extracellular dialysate concentrations of 5HIAA, dopamine or GABA of the sucrose group and the starch group (Mann-Whitney's test, $\alpha = 0{\cdot}05$).

Table 1. Basal extracellular hippocampal concentrations of 5-hydroxytryptamine (5HT), dopamine, γ-aminobutyric acid (GABA) and 5-hydroxyindoleacetic acid (5HIAA) of freely-moving rats fed with regular food–starch (starch group) and regular food–sucrose (sucrose group)†

(Mean values with their standard errors)

	Starch group			Sucrose group		
	Mean	SEM	*n*	Mean	SEM	*n*
EC 5HT (nM)	0·396	0·097	5	2·332*	0·766	7
EC dopamine (nM)	0·265	0·050	7	0·236	0·051	7
EC GABA (μM)	0·043	0·008	7	0·058	0·014	7
EC 5HIAA (μm)	0·296	0·030	5	0·278	0·032	7

EC, extracellular.
Mean value was significantly different from corresponding baseline value in starch group: *$P < 0{\cdot}05$.
† For details of diets and procedures, see p. 152.

The major finding of the present study is that the baseline extracellular hippocampal 5HT levels of the freely-moving rats with access *ad libitum* to food supplemented with sucrose were significantly higher compared with the concentrations obtained in the starch control group. In our experiments, rats were subjected to chronic sucrose supplementation and had free access to their food mixture, a metabolic situation truly different from the one in animals undergoing sudden alterations in the value of the tryptophan:large neutral amino acids ratio due to short-lasting carbohydrate consumption (Young, 1991) or from a food-deprived state (Reaven & Ho, 1991). Thibault (1994) and Young & Landsberg (1977), however, used comparable experimental conditions as in the present study (i.e. chronic feeding, access *ad libitum*). Chronic sucrose feeding significantly increased blood insulin concentrations (Thibault, 1994). Moreover, sucrose elevated the insulin levels clearly more than glucose, starch or fructose. Since elevated plasma insulin leads to the uptake of glucose and branched-chain amino acids into muscles, it is possible that during chronic sucrose feeding the value of the plasma tryptophan:large neutral amino acid ratio did increase as well and hence resulted in augmented brain tryptophan levels. Young & Landsberg (1977) showed that chronic sucrose feeding significantly stimulates sympathetic activity. Since increased sympathetic activity, mediated *via* β-adrenergic receptors, is known to raise brain tryptophan levels (Eriksson & Carlsson, 1982; Eriksson *et al.* 1984; Edwards *et al.* 1989), this could be another mechanism by which chronic sucrose feeding significantly affected brain serotoninergic systems. Importantly, these data also imply that dietary sucrose supplementation might bias the outcome of studies investigating brain serotoninergic mechanisms.

Monitoring alterations in hippocampal 5-hydroxytryptamine and 5-hydroxyindoleacetic acid release after intraperitoneal injection of S(+)fenfluramine

Fig. 1 shows that the intraperitoneal administration of S(+)fenfluramine (1 mg/kg) resulted in significant elevations of the extracellular 5HT levels, both in the sucrose group ($P = 0{\cdot}001$) and the starch group ($P = 0{\cdot}0001$) (ANOVA and Fisher's protected least significant difference *post hoc* test). We showed that the absolute values of the sucrose response curve remained higher than in the starch group throughout. Nevertheless, the extracellular 5HT concentrations in the starch group remained significantly elevated until the end of the microdialysis experiment, while in the sucrose group baseline levels were already attained within the 2 h following S(+)fenfluramine injection. In the collection period that immediately followed S(+)fenfluramine injection (collection period 14), a relative maximum increase in the extracellular 5HT levels up to 221 % was observed in the sucrose group. In the starch group, peak dialysate concentrations of 5HT were

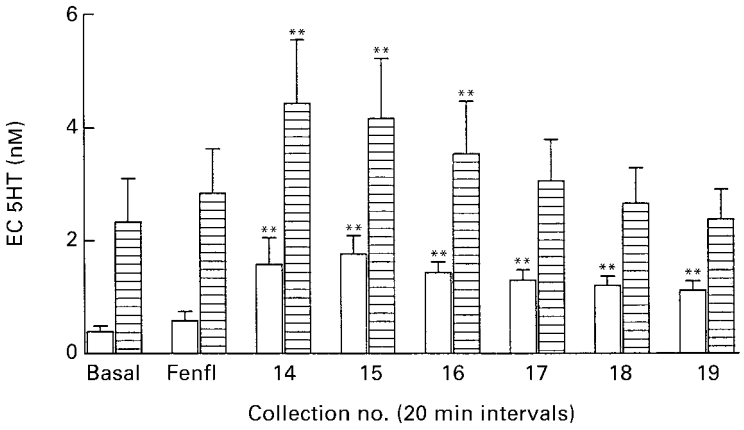

Fig. 1. Hippocampal dialysate concentrations of 5-hydroxytryptamine (5HT) in rats fed with regular food – starch (□ starch group, *n* 5) or regular food–sucrose (▤ sucrose group, *n* 7), before (basal) and following intraperitoneal injection (1 mg/kg) of S(+)fenfluramine (fenfl). For details of diets and procedures, see p. 152. EC, extracellular. Values are means with standard errors of the means shown by vertical bars. The baseline value is the mean of the stable 5HT dialysate concentrations before administration of fenfl. Each following value represents a 20 min collection period. Mean values were significantly different from the corresponding baseline values: $^{**}P < 0{\cdot}01$ (one-way ANOVA for repeated measures, and Fisher's protected least significant difference *post hoc* test, $\alpha = 0{\cdot}05$.)

reached in collection period 15, up to 474 % baseline 5HT level. At all time-points, the extracellular 5HT dialysate levels, expressed as a percentage of the baseline value, were significantly different between the two experimental groups (Mann-Whitney, $P < 0{\cdot}05$). Extracellular hippocampal 5HIAA concentrations were significantly diminished by 15 % following S(+)fenfluramine injection (ANOVA and Fisher's protected least significant difference *post hoc* test, $P = 0{\cdot}0001$) but there was no statistical difference between the two experimental groups (results not shown).

Acute intraperitoneal injection of S(+)fenfluramine (1 mg/kg) resulted in a significant elevation of the extracellular 5HT levels in the hippocampus of rats of both groups. Simultaneously, we recorded significant decreases in the extracellular hippocampal 5HIAA levels of both groups following S(+)fenfluramine administration. These data confirm previous studies in which, however, higher doses of the drug were used. Acute injection of S(+)fenfluramine (10 mg/kg) increased 5HT release and diminished 5HIAA concentrations in the frontal cortex, lateral hypothalamus and nucleus accumbens. In these three brain regions, 5HT concentrations peaked 30 min after drug administration and returned to baseline levels within 60–100 min, while 5HIAA levels were decreased by 30 % (Laferrere & Wurtman, 1989). Systemically administered S(+)fenfluramine (3 and 10 mg/kg) dose-dependently increased extracellular levels of 5HT for about 60–80 min and consistently decreased extracellular 5HIAA concentrations in the perifornical lateral hypothalamus of freely-moving rats (Schwartz *et al.* 1989). An acute injection of 12·5 mg S(+)fenfluramine/kg also caused large increases in extracellular hippocampal 5HT levels (Sabol *et al.* 1992). Increases in extracellular 5HT evoked by 5HT-re-uptake-inhibiting–5HT-releasing substances are presumed to result first from 5HT re-uptake inhibition and then, mostly at higher doses, from increased release *per se* (Garattini *et al.* 1992; Fuller, 1994). This implies that the increases in the 5HT levels shown in the present study are likely to be due to 5HT re-uptake blockade rather than 5HT release. The decreases in extracellular 5HIAA in both the sucrose and starch group are consequently the result of a diminished cytoplasmic pool of 5HT available for catabolisation by monoamine oxidase. The decrease in cytoplasmic 5HT will result in a smaller amount of metabolite released into the extracellular space (Sarre *et al.* 1997).

Interestingly, the sucrose supplementation affected the dynamics of the S(+)fenfluramine-induced hippocampal 5HT release, as compared with the starch group. We observed that the absolute values of the sucrose response curve remained higher than in the starch group throughout the experiment, but the effects in the starch group were relatively more expressed and longer lasting. It is generally known that the peripheral metabolism of starch is much slower in comparison to that of sucrose, which may partly explain the altered dynamics of 5HT release after S(+)fenfluramine injection. Indeed, it is possible that a peripheral post-absorptive mechanism is involved since the present situation is clearly different from the acute carbohydrate-induced effects immediately following ingestion (Rouch *et al.* 1999). Nevertheless, other phenomena might also be involved.

Acknowledgements

I. Smolders and S. Sarre are postdoctoral research fellows of the Fund for Scientific Research Flanders (FWO-Vlaanderen, Belgium). We are most obliged to ORAFTI (Tienen, Belgium), the FWO-Vlaanderen, the Vrije Universiteit Brussel and the Koningin Elisabeth Stichting for

financial support. The authors appreciate the excellent technical assistance provided by Mrs R. Berckmans, Mr G. De Smet, Mrs C. De Rijck and Mrs R. M. Geens.

References

Blundell JE & Halford JCG (1998) Serotonin and appetite regulation. Implications for the pharmacological treatment of obesity. *CNS Drugs* **9**, 473–495.

Caccia S, Confalonieri S, Bergami A, Fracasso C, Anelli M & Garattini S (1997) Neuropharmacological effects of low and high doses of repeated oral dexfenfluramine in rats: a comparison with fluoxetine. *Pharmacology, Biochemistry and Behavior* **57**, 851–856.

Carboni E, Cadoni C, Tanda GL & Di Chiara G (1989) Calcium-dependent, tetrodotoxin-sensitive stimulation of cortical serotonin release after a tryptophan load. *Journal of Neurochemistry* **53**, 976–978.

Edwards DJ, Sorisio DA & Knopf S (1989) Effects of the beta 2-adrenoreceptor agonist clenbuterol on tyrosine and tryptophan in plasma and brain of the rat. *Biochemical Pharmacology* **38**, 2957–2965.

Eriksson T & Carlsson A (1982) Isoprenaline increases brain concentrations of administered L-DOPA and L-tryptophan in the rat. *Psychopharmacology (Berl)* **77**, 98–100.

Eriksson T, Carlsson A, Hagman M & Jagenburg R (1984) β-Adrenergic influence on brain concentrations of monoamine precursors. *Advances in Biochemical Psychopharmacology* **39**, 263–270.

Fuller RW (1994) Uptake inhibitors increase extracellular serotonin concentration measured by brain microdialysis. *Life Sciences* **55**, 163–167.

Garattini S, Bizzi A, Caccia S & Mennini T (1992) Progress report on the anorectic effects of dexfenfluramine, fluoxetine and sertraline. *International Journal of Obesity and Related Metabolic Disorders* **16**, Suppl. 3, S43–S50.

Laferrere B & Wurtman RJ (1989) Effect of D-fenfluramine on serotonin release in brains of anaethetized rats. *Brain Research* **504**, 258–263.

Leathwood PD (1987) Tryptophan availability and serotonin synthesis. *Proceedings of the Nutrition Society* **46**, 143–156.

Paxinos G & Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd ed., San Diego, CA: Academic Press Inc.

Reaven GM & Ho H (1991) Sugar-induced hypertension in Sprague-Dawley rats. *American Journal of Hypertension* **4**, 610–614.

Rouch C, Nicolaidis S & Orosco M (1999) Determination, using microdialysis, of hypothalamic serotonin variations in response to different macronutrients. *Physiology & Behavior* **65**, 653–657.

Sabol KE, Richards JB & Seiden LS (1992) Fenfluramine-induced increases in extracellular hippocampal serotonin are progressively attenuated in vivo during a four-day fenfluramine regimen in rats. *Brain Research* **571**, 64–72.

Sarre S, Marvin CA, Ebinger G & Michotte Y (1992) Microbore liquid chromatography with dual electrochemical detection for the determination of serotonin and 5-hydroxyindoleacetic acid in rat brain dialysates. *Journal of Chromatography* **582**, 29–34.

Sarre S, Smolders I, Thorré K, Ebinger G & Michotte Y (1997) Biotransformation of locally applied precursors of dopamine, serotonin and noradrenaline in striatum and hippocampus: a microdialysis study. *Journal of Neural Transmission* **104**, 1215–1228.

Schwartz DH, Hernandez L & Hoebel BG (1989) Fenfluramine administered systemically or locally increases extracellular serotonin in the lateral hypothalamus as measured by microdialysis. *Brain Research* **482**, 261–270.

Schwartz DH, Hernandez L & Hoebel BG (1990) Tryptophan increases extracellular serotonin in the lateral hypothalamus of food-deprived rats. *Brain Research Bulletin* **25**, 803–807.

Sharp T, Bramwell SR & Grahame-Smith DG (1992) Effect of acute administration of L-tryptophan on the release of 5HT in rat hippocampus in relation to serotoninergic neuronal activity: an in vivo microdialysis study. *Life Sciences* **50**, 1215–1223.

Smolders I, Sarre S, Ebinger G & Michotte Y (1995) The analysis of excitatory, inhibitory and other amino acids in rat brain microdialysates using microbore liquid chromatography. *Journal of Neuroscience Methods* **57**, 47–53.

Smolders I, Sarre S, Vanhaesendonck C, Ebinger G & Michotte Y (1996) Extracellular striatal dopamine and glutamate after decortication and kainate receptor stimulation, as measured by microdialysis. *Journal of Neurochemistry* **66**, 2373–2380.

Stancampiano R, Melis F, Sarais L, Cocco S, Cugusi C & Fadda F (1997) Acute administration of a tryptophan-free amino acid mixture decreases 5HT release in rat hippocampus in vivo. *American Journal of Physiology* **272**, R991–R994.

Thibault R (1994) Dietary carbohydrates: effects on self-selection, plasma glucose and insulin, and brain indoleaminergic systems in rat. *Appetite* **23**, 275–286.

van Kempen GMJ (1994) 5HT: Biochemistry, physiology and metabolism. In *Serotonin (5HT) in Neurologic and Psychiatric Disorders*, pp. 1–12 [MD Ferrari, GMJ van Kempen, PR Saxena and PR Bär, editors]. Leiden: Boerhaave Commissie voor Postacademisch Onderwijs in de Geneeskunde.

Wurtman RJ & Wurtmann JJ (1995) Brain serotonin, carbohydrate-craving, obesity and depression. *Obesity Research* **3**, Suppl. 4, 477S–480S.

Young JB & Landsberg L (1977) Stimulation of the sympathetic nervous system during sucrose feeding. *Nature* **269**, 615–617.

Young SN (1991) The 1989 Borden Award Lecture. Some effects of dietary components (amino acids, carbohydrate, folic acid) on brain serotonin synthesis, mood and behavior. *Canadian Journal of Physiology and Pharmacology* **69**, 893–903.