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

Ilse Smolders¹, Jan Van Loo², Sophie Sarre¹, Guy Ebinger³ and Yvette Michotte¹*

¹Department of Pharmaceutical Chemistry and Drug Analysis, Pharmaceutical Institute, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium
²ORAFTI, Aandorenstraat 1, 3300 Tienen, Belgium
³Department of Neurology, University Hospital, Laarbeeklaan 101, 1090 Brussels, Belgium

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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 serotonergic 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,β-D-fructofuranosyl-β,γ-D-glucopyranosyl-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 CaCl2 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-MCl, 2 g sodium metabisulphite/l and 0.2 g Na2EDTA/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 derivatization (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.
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, α = 0.05). 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, α = 0.05). The significance of differences between peak dialysate levels was tested by Mann-Whitney’s test (two-tailed, α = 0.05).

### 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, α = 0.05). 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 = 0.024) 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, α = 0.05).

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 = 0.001) and the starch group (P = 0.0001) (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.

### 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)†

<table>
<thead>
<tr>
<th>Starch group</th>
<th>Sucrose group</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>EC 5HT (nM)</td>
<td>0.396</td>
</tr>
<tr>
<td>EC dopamine (nM)</td>
<td>0.265</td>
</tr>
<tr>
<td>EC GABA (μM)</td>
<td>0.043</td>
</tr>
<tr>
<td>EC 5HIAA (μM)</td>
<td>0.296</td>
</tr>
</tbody>
</table>

EC, extracellular.

Mean value was significantly different from corresponding baseline value in starch group: *P < 0.05.

† For details of diets and procedures, see p. 152.
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, \( P < 0.05 \)). Extracellular hippocampal 5HIAA concentrations were significantly diminished by 15 % following \( S(+) \)fenfluramine injection (ANOVA and Fisher’s protected least significant difference post hoc test, \( P = 0.0001 \)) 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 % (Laferriere & 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.

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