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Behaviour and hormonal status in healthy rats on a diet rich in Maillard reaction products with or without solvent extractable aroma compounds

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ABSTRACT

Maillard reaction products (MRPs) are generated upon thermal processing of foods, modifying their colour and flavour. We asked whether aroma compounds generated *via* Maillard-type reactions modulate the *in vivo* effects of MRP-rich diets (MRPD). Male Wistar rats were fed for 3 weeks either with a standard rat chow, an aroma compounds containing MRPD comprising 25% bread crust, or an aroma-extracted MRPD. In contrast to standard rat chow, consumption of MRPDs affected glucose control, induced hyper-leptinemia and hyper-adiponectinemia. Plasma adipokines were significantly higher in rats on aroma containing MRPD in comparison with those consuming aroma-extracted MRPD. Consumption of both MRPDs significantly increased the expression of the insulin receptor in the olfactory bulb, and mildly in the hypothalamus. Administration of the aroma containing MRPD significantly increased the leptin receptor expression in the olfactory bulb, and in the hypothalamus. Under both MRPDs, strong expression of *c-fos* indicated an increased neuronal activity in the olfactory bulb. Neuronal activity in brain areas involved in the central regulation of food intake and energy homeostasis was more pronounced in rats fed by the aroma containing MRPD. In conclusion, short-term consumption of a MRPD fortified with bread crust, particularly if containing solvent extractable volatile aroma compounds, affected the leptin-induced central signalling of anorexigenic/orexigenic hormones, and the neuronal activity in the central nervous system. Behavioural changes and altered glucose control were more evident in rats on the aroma containing MRPD. Our data suggest that volatile aroma compounds in foods might affect endocrine signalling and neuronal regulation of metabolism.

1. Introduction

Maillard reaction products (MRPs) are generated *via* non-enzymatic reactions between reducing sugars and proteins. In foods, high contents of MRPs are formed upon thermal processing. The diversity of reacting proteins and sugars renders the characteristic colour and flavour of processed foods.

The complex mechanism of chemosensory olfactory perception recognizes volatile aroma compounds with great accuracy and sensitivity. Taste and smell are interconnected: 80–90% of what is perceived as food taste is due to the sense of smell. The flavour of foods is one of the most important factors that determine the quantity and frequency of food consumption, since aroma compounds, even at very low concentrations, are accurately and long-lastingly remembered [1,2].

Although more than 280 compounds have been identified in the volatile fraction of wheat bread, only a small number is responsible for the flavour notes in the crust and the crumb [3]. The aroma extract dilution analysis, followed by identification experiments, revealed the following flavour compounds that are extractable by dichloromethane or diethylether and act as contributors to the overall bread crumb flavour: 3-methylbutanal (malty), (E)-2-nonenal (green, fatty), (E,E)-2,4-decadienal (fatty, waxy), hexanal (green), acetic acid (sour, pungent), phenylacetaldehyde (honey-like), methional (boiled potato-like), vanillin (vanilla-like), 2,3-butandione (buttery), 3-hydroxy-4,5-dimethyl-2(5H)-furanone (spicy), and 2- and 3-methylbutanoic acid (sweaty), [4–6]. This list,

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however, is not complete, although it comprises the major aroma compounds in bread crust.

Food-related odours have been shown to increase or decrease appetite, and to affect behaviour by enhancing the feeling of comfort, or, by evoking distress [7,8]. Once an odour is experienced in a food-related context, the odour acquires the ability to modify both preparatory and satiety-related components of ingestion [8,9]. In rats, the end of ingestion is accompanied by characteristic "satiety sequence": grooming, exploring, and followed by resting [10]. Among the aroma compounds present in bread crusts, 3-methylbutanal has been shown to increase the brain serotonin concentration, and induce sleep-like behaviour in rats [11]. In contrast, vanillin did not affect rat feeding behaviour adversely [12]. According to our best knowledge, it is not known whether and how complex MRP-derived bread crust flavours, or their absence, affect behaviour of rats.

Dietary MRPs are at least partially absorbed into circulation, and long-term exaggerated intake of highly thermally processed foods rich in MRPs exerts harmful effects, *e.g.* diabetogenic and nephrotoxic effects, accelerated atherosclerosis, or activation of inflammatory and oxidative stress pathways [13,14]. MRPs as well as advanced glycation end products (AGEs, *in vivo* analogues to MRPs), may affect insulin sensitivity and induce insulin resistance in skeletal muscle and adipocytes [15–17]. In humans, high intake of dietary MRPs is associated with decreased insulin sensitivity, and circulating AGE levels are directly related to insulin resistance [18–21]. As far as metabolic effects of aroma compounds are concerned, for 5-methyl and 6-methylpyrazinoic acid and their metabolite 2-furoic acid, a triacylglycerol-lowering effect in normolipidemic rats was demonstrated [22]. Whether bread-crust-derived volatile aroma MRPs exert metabolic effects is still unknown.

Thus, olfactory signals from volatile MRPs conveyed *via* the olfactory bulb to distinct brain areas may elicit not only emotional and behavioural, but also metabolic responses. We aimed to elucidate the contribution of volatile aroma compounds to these effects of an MRP-rich diet (MRPD). In this study, healthy rats were fed either a diet enriched with bread crust (aroma-containing MRPD), or bread crust subjected to solvent-extraction to remove volatile aroma compounds (aroma-extracted MRPD), while control rats consumed a standard rat chow. Effects of these diets on behaviour, and signalling related to satiety were investigated for the first time.

2. Material and methods

2.1. General design

The study was conducted according to the guidelines for experimental studies using laboratory animals (86/609/EEC), after approval by the State Veterinary and Food Control Agency in Bratislava (Slovakia). Two animals per cage were held in a room with constant temperature and humidity, 12 h/12 h light cycles (inversed to daylight), and had *ad libitum* access to tap water. During the adaptation period (1 week), animals consumed a standard rat chow (SP1, Top Dovo, Slovakia) *ad libitum*.

Eight weeks old male Wistar rats were obtained from Charles River, Hungary. After adaptation, they were randomized into three groups (each n = 8) with comparable body weight and proteinuria and were administered one of the following diets *ad libitum* for three weeks: 1/control diet (un-pelleted standard rat chow/starch, 80:20 wt/wt); 2/aroma-extracted MRPD, containing solvent extracted bread crust, standard rat chow and starch, 25:55:20 wt/wt, respectively; and 3/volatile aroma-containing MRPD (aroma-containing bread crust, standard rat chow and starch, 25:55:20 wt/wt). Bread crusts from German sourdough bread were prepared as described previously [23]. Energy content of the standard rodent diet was 290 kcal/100 g, and that of the bread crusts 390 kcal/100 g. Solvent extracted bread crust was prepared by triplicate extraction of 500 g of bread crust with 1500 ml of diethylether for 6 h per extraction step. After evaporation of diethylether, the residue was stored at −80 °C until the experiment. The mentioned procedure of solvent extraction eliminates aroma compounds [5,6].

Food and water consumption were recorded daily, and expressed per individual animal. Rats were placed into metabolic cages for fasting, stool-free 24-hour urine collection before the initiation, and at the end of the experiment. At day 15 (after 24 h fasting), rats underwent an oral glucose tolerance test (oGTT): glucose was administered by gavage (2 g glucose/1 kg body weight). Before gavage and 1 h thereafter, blood was taken under anaesthesia (Isoflurane, Abbott, UK) from the tail vein. Surrogates (HOMA index based on fasting glucose and insulin, simple 2-point oGTT, and HbA1), accepted in experimental settings in rodents in cases when use of clamps is an obstacle [24], were used to characterize glucose metabolism.

Before sacrifice, rats were not deprived of food. At sacrifice, blood samples for biochemistry were collected from the abdominal aorta under i.p. ketamin/xylazin anaesthesia. Brains (4 rats/group) were removed, dissected, and isolated olfactory bulbs and hypothalami were frozen in liquid nitrogen and stored at −80 °C, until RNA isolation. Remaining rats (4/group) were assigned for analysis of brains c-Fos immunohistochemistry. Rats were cannulated through the left ventricle and right carotid artery, the brain was perfused by chilled saline, and fixed by infusion of 4% paraformaldehyde. Brains were removed and postfixed in the same fixative solution and stored until further processing. Organs (liver, kidney, brain) were weighed.

2.2. Biochemical analyses

Standard blood plasma and urine parameters were measured (Vitros 250 analyzer, J&J, Rochester, USA). Plasma advanced oxidation protein products (AOPPs) [25] and advanced glycation end-products specific fluorescence (AGE-Fl) [26] were determined. Proteinuria was determined by the pyrogallol red method. Plasma C-reactive protein (CRP) and leptin (ELISA, Biovendor Laboratory Medicine Inc., Modrice, Czech Republic), insulin and adiponectin (RIA, LINCO Research, Missouri, USA) were analysed using rat specific commercially available kits. Assays were performed according to manufacturers' instructions with following intra-assay coefficients of variation: CRP: 5.2%; leptin: 5.7%; insulin: 4.3%; and adiponectin: 3.8%. Insulin sensitivity was evaluated using the homeostasis model assessment index (HOMA). Postprandial glucose tolerance was estimated using insulin to glucose ratio. Haemoglobin A1c concentrations were determined by routine laboratory HPLC method.

2.3. Quantitation of N^{ε}-(carboxymethyl)lysine (CML)

CML concentrations in plasma samples were analysed as described previously [14]. Briefly, aliquots of plasma were reduced using $NaBH_4$. Following reduction, proteins were precipitated by adding trichloroacetic acid. To each sample, stable isotope labelled internal standards of [^{13}C2]-CML and [^{13}C6,^{15}N2]-lysine were added. Afterwards, protein hydrolysis was performed by adding hydrochloric acid. After hydrolysis, hydrochloric acid was removed by centrifugal evaporation. Hydrolysates were rehydrated in trifluoroacetic acid and applied to a solid phase extraction column. The eluates were dried by centrifugal evaporation and derivatised for GC/MS analysis into N,O-trifluoroacetyl methyl ester derivatives (TFAME). Methyl esters were prepared by adding methanolic HCl. The solvent was evaporated under a stream of nitrogen. Then, trifluoroacetic anhydride was added and the mixture was incubated at room temperature. The derivatised samples were again dried under nitrogen, reconstituted in methylene chloride, centrifuged and analysed by SIM-GC/MS carried out on a 6890 Network GC system (Agilent Technologies, Waldbronn, Germany). The ions monitored were m/z 320 and 328 for lysine and [^{13}C6,^{15}N2]-lysine, m/z 392 and 394 for CML and [^{13}C2]-CML, respectively. Quantification was based on external standardization. Because the aliquot of the