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Do taste receptors respond to perturbation of water structure?

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Abstract. The pmr spin-spin pulse relaxation times (T_2 values) of the L-amino acids are examined in relation to their taste threshold values. There is an inverse trend between T_2 value and threshold value with a good correlation for amino acids whose natural pH is close to neutrality. These results may indicate that taste receptors respond to perturbation of water structure.

Recent interest in the solution properties of small carbohydrate molecules in relation to their sweet taste[1,2] and indeed, in the 'packing characteristics' of all sapid molecules in water as predictors[3] of their taste quality, has refocussed attention on water structure[4–7]. A heavily hydrated solute, such as a sugar or an amino acid is likely to interfere, substantially, with hydrogen-bonding between water molecules, and this process may be of particular significance in the localised micro-environment of receptors, where water activity is minimal[1,3]. The response of a receptor to water structure perturbation, rather than to stimulus molecular structure might be manifested more in terms of detection threshold (see ref. 17 below), than recognition threshold or supra-threshold magnitude estimation, because the low concentration of stimulus involved avoids the complexity of recognition and memory processes.

Materials and methods

T_2 values were determined using a Bruker Minispec NMR Spectrometer, a low resolution instrument (20 MHz) operating at 40 °C. The Carr-Purcell, Meiboom Gill (CPMG) sequence was used to measure T_2 values. The T_2 values thus obtained reflect the exchange of spin energy between protons which in turn depends on their structural environment within the molecules. Amino acids were reagent grade chemicals obtained from Sigma Chemical Company, Poole, Dorset, except for L-alanine, L-glutamic acid L-histidine and L-leucine which were obtained from BDH, Poole, Dorset. Water used was 'Water for HPLC' (BDH).

Results and discussion

The table ranks fourteen L-amino acids in descending order of pmr spin-spin relaxation times (T_2 values) and again in ascending order of detection thresholds. The two rankings are not identical but similar. The T_2 ranking reflects differences in overall energy exchange between water protons, and in this respect is an indicator of the solute's influence on water structure.

The table lists the natural pHs for the amino acid solutions. This demonstrates that amino acids with a high or low pH have the lowest detection thresholds. T_2, which is dependent upon pH (figs 1 and 2), differentiates these amino acids. High or low pH would of course distinguish

Ranking of the L-amino acids according to spin-spin relaxation times, T_2 at a 2-ms 180° pulse separation and detection threshold values Cd.

Cd[17] (mM/l)	pH	T_2 (sec)
Glu 0.063	3.4	Arg 3.9
Lys 0.708	9.6	Lys 3.8
Arg 1.20	10.1	Glu 3.3
His 1.23	7.3	His 3.2
Trp 2.29	6.0	Trp 3.2
Met 3.72	5.8	Pro 3.1
Val 4.16	6.1	Met 3.0
Leu 6.45	5.9	Phe 3.0
Phe 6.61	6.3	Val 2.9
Ile 7.14	6.4	Leu 2.9
Gln 9.77	5.7	Gln 2.9
Pro 15.1	6.4	Ile 2.9
Ala 16.2	5.9	Ala 2.9
Ser 20.9	6.3	Ser 2.8

The T_2 values were measured on a proton magnetic resonance spectrometer operating at a frequency of 20 MHz and at a sample temperature of 40 °C. The CPMG sequence was used to measure T_2[18].

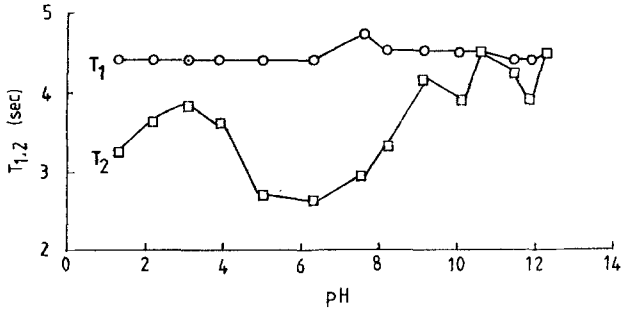

Figure 1. T_2 and T_1 plotted as a function of pH for 0.6% w/w L-arginine in aqueous solution. T_1 is measured by the inversion recovery method.

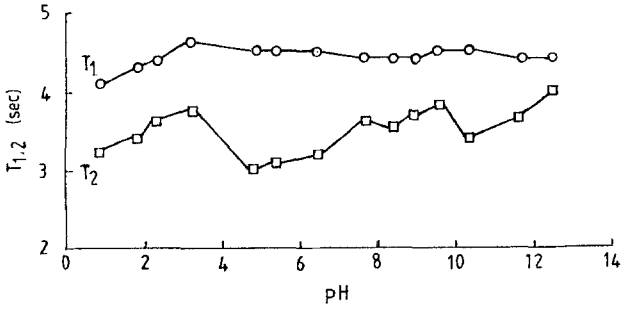

Figure 2. T_2 and T_1* plotted as a function of pH for 0.6% w/w L-lysine in aqueous solution. * T_1 is the pmr spin-lattice relaxation time.

some amino acids. However, what is more interesting is the ability of T_2 to differentiate the detection thresholds of amino acids at pH near neutrality. Work by Hertz and coworkers[8] has directed interest in respect to the exchange rate of protons. This parameter differentiated structure making and structure breaking salts in regard to the way that the salt disrupts water hydrogen bonds. In the same regard T_2 of the amino acid aqueous solutions reflects disturbance of water structure. Therefore, ignoring glutamic acid, lysine and arginine, T_2 is related to detection thresholds with a linear regression, where $r = 0.551$.

Proline seems odd in respect to the trend. Past research carried out at this laboratory has shown that the NMR response for proline in aqueous solution is not easy to predict[9] and this may demonstrate that proline has an unusual interaction with water. If proline is ignored in the relationship between T_2 and detection thresholds, a linear regression where $r = 0.729$ is achieved.

If this experiment had been conducted at higher concentrations, the recognition thresholds or supra-threshold determinations of taste would have been confused by the sweet and/or bitter response elicited by the amino acids, depending on their structure, molecular weight and chirality[10]. Indeed, the amino acids resemble many sugar derivatives in eliciting both sweet and bitter tastes and this lends support to the idea that the two basic tastes are closely associated, chemically, anatomically and psychophysically. The thresholds listed in the table represent a response which is detectable at a concentration below that at which the taste quality of the stimulus molecule is perceivable. This is indicative of interaction of the taste receptor with water itself.

Water alone is recognised to possess a 'taste', the nature of which depends on pre-adaption conditions[11]. Indeed any of the four basic taste qualities (sweet, sour, salty, and bitter) may be elicited by water if the pre-adapting solution is appropriately selected, though the most easily elicitable quality is sweetness[12]. However, randomly presented salt and water solutions are discriminated by panellists with varying degrees of success, depending on the order of presentation, the best discriminable being salt preceded by water and next water preceded by salt[13]. Water is less discriminable from salt if immediately preceded by water and salt preceded by salt is least discriminable. These results (conducted with 3 mM NaCl solutions) of course show that the receptors respond best to change but they are also supportive of the idea that the change from least disturbed to most disturbed solutions is the most discriminable. Actually the threshold concentration of sodium chloride falls[14] from 5.8 mM when 0.05-ml samples are tested to 2.8 mM with 0.5-ml samples. Thus if it is perturbation of water structure which is responsible for the response, a threshold number of receptors needs to be stimulated. At supra-threshold levels of stimulation by ionic solutes it is not known whether anion or cation or both interact with receptors. With detection thresholds, however, it seems more likely that the overall perturbation of water structure (depending on ionic strength) may be responsible for stimulation and a recently demonstrated[15] correlation between detection threshold and molar conductivity of a series of sodium salts provides more support for this idea. The interesting correlation between detection threshold and T_2 values of the amino acids listed in the table underlines the role of hydrogen bonding in both water structure and taste chemoreception[16] as the hydrogen bond provides the channel[8] by which protons exchange energy during the nmr relaxation processes.

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Gastrointestinal transit and digestibility of maltitol, sucrose and sorbitol in rats: a multicompartmental model and recovery study

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Abstract. Using data obtained with a dye marker and the gavage technique, the kinetics of gastrointestinal transit of different loads of sugar substitutes (maltitol, sorbitol) and sugar (sucrose) in the rat were analysed using a linear multicompartmental model over a range from the realistic to the non-physiologic high, of carbohydrate intake levels and using only a few experimental time points. The model gave detailed insight into intestinal propulsion and gastrocecal transit time. Rate constants of transport between the compartments investigated were determined; they showed characteristics which could be related to the substance and the dosage administered. Analyses of the gastrointestinal content and calculations of the intestinal net water movement showed that the digestibility and absorption of the disaccharide sugar alcohol, maltitol, in the small gut depended inversely on the dose ingested. For all substances tested, caloric availability in the small intestine was calculated. At a physiological low level of maltitol intake, the results also indicated an insignificant calorie-saving effect in comparison to sucrose, an effect based mainly on the slow absorption rate of the maltitol cleavage product sorbitol.
Key words. Linear multicompartmental model; gastrointestinal tract; sugar substitutes; maltitol; sucrose; sorbitol; absorption; small intestine; caloric value.

It is of physiological and nutritional interest to characterize the gastrointestinal transit of digesta in humans and animals[1]. Several methods are available to measure the digestive flow through distinct intestinal regions[2–5], and attempts have been made to describe this mathematically[6–8].

The rate of intestinal passage is influenced by various factors[9,10] and has physiological consequences. For instance, increased orocecal transit time was shown to be closely related to carbohydrate malabsorption in the small intestine[11,12].

This may be of importance when investigating the caloric availability of alternative carbohydrates, e.g. the sugar substitutes; they are known to influence the gastrointestinal transit time by exerting dose-related osmotic effects[13,14].

Estimates of the calories available from a new sugar substitute of the disaccharide sugar alcohol type, i.e. maltitol (D-glucopyranosido-1,4-sorbitol), exist for humans and animals[15,16]. A reduction of caloric utilization of up to 50% of that of the normal dietary carbohydrate sucrose (D-glucopyranosido-1,2-fructofuranosid, 4 kcal/g) has been suggested[17,18]. However, the results concerning the fractions of maltitol cleaved and absorbed in the small intestine, or transformed by the colonic microflora to absorbable fermentation acids, are contradictory[15,16,19,20].

In vitro studies using small intestinal disaccharidases from rats and maltitol have questioned the postulated low physiologic calorie yield[21]. In vivo studies with different animal species suggested that the slow rate of absorption of the sorbitol moiety of maltitol is the decisive mechanism limiting its availability via the small intestine[22,23]. Feeding experiments with rats (including isocaloric starch replacement) revealed a significantly retarded weight increase in the group fed with maltitol[24]. However, the latter study, like most former experiments, involved introducing huge, non-physiological amounts of maltitol; such conditions are obviously unsuited for estimating the caloric availability of disaccharide sugar alcohols at a realistically low intake[13].

Therefore, this study aimed to develop a compartmental model describing the gastrocecal transit and the sites of absorption of different doses of sugar and sugar substitutes. Based on experimental data obtained with rats and using a dye marker to determine intestinal transit, the dose range screened included small-, medium- and non-physiological high doses of the tested carbohydrates. Ad-