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(+)-(*S*)-Alapyridaine—A General Taste Enhancer?

Tomislav Soldo, Imre Blank[1] and Thomas Hofmann[2]

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany, [1]Nestlé Research Center, Vers-chez-les-Blanc, PO Box 44, 1000 Lausanne 26, Switzerland and [2]Institut für Lebensmittelchemie, Universität Münster, Corrensstrasse 45, D-48149 Münster, Germany

Correspondence to be send to: Thomas Hofmann, Institut für Lebensmittelchemie, Universität Münster, Corrensstrasse 45, D-48149 Münster, Germany. e-mail: thomas.hofmann@uni-muenster.de

Abstract

N-(1-Carboxyethyl)-6-hydroxymethyl-pyridinium-3-ol inner salt (alapyridaine), recently identified in heated sugar/amino acid mixtures as well as in beef bouillon, has been shown to exhibit general taste-enhancing activities, although tasteless on its own. Differing from other taste enhancers reported so far, racemic (*R/S*)-alapyridaine and, to an even greater extent (+)-(*S*)-alapyridaine, the physiologically active enantiomer, are able to enhance more than one basic taste quality. The threshold concentrations for the sweet taste of glucose and sucrose, for the umami taste of monosodium L-glutamate (MSG) and guanosine-5′-monophosphate (GMP), as well as the salty taste of NaCl, were significantly decreased when alapyridaine was present. In contrast, perception of the bitter tastes of caffeine and L-phenylalanine, as well as of sour-tasting citric acid, was unaffected. Furthermore, alapyridaine was shown to intensify known taste synergisms such as, for example, the enhancing effect of L-arginine on the salty taste of NaCl, as well as that of GMP on the umami taste of MSG. The activity of (+)-(*S*)-alapyridaine could be observed not only in solutions of single taste compounds, but also in more complex tastant mixtures; for example, the umami, sweet and salty taste of a solution containing MSG, sucrose, NaCl and caffeine was significantly modulated, thus indicating that alapyridaine is a general taste enhancer.

Key words: alapyridaine, taste enhancer, umami taste, sweet taste, salty taste, *N*-(1-carboxy-ethyl)-6-hydroxymethyl-pyridinium-3-ol

Introduction

The sensory impression of flavor is due to the simultaneous stimulation of the human olfactory and taste systems and is triggered by chemical compounds in food products. Although the overall flavor and, in consequence, the consumer acceptance of foods is strongly influenced by the interplay of aroma-active volatiles, taste-active non-volatiles and flavor modifiers enhancing or suppressing certain taste qualities, flavor research in recent decades has focused mainly on aroma and taste compounds, rather than on compounds that are tasteless on their own, but that show synergistic effects with basic taste compounds.

More than 40 years ago, the first flavor synergism was reported between the umami-like tasting monosodium L-glutamate (MSG) and purine-5′-ribonucleotides and analogues of both groups (Kuninaka, 1967; Yamaguchi, 1967; Yamaguchi *et al.*, 1971). Systematic sensory studies on the umami taste of binary mixtures of MSG and inosine-5′-monophosphate (IMP) varying in the concentration ratio revealed an exponential increase in the umami-like taste intensity of MSG even when IMP (1–12% based on MSG) was added in low concentrations only (Yamaguchi, 1967). Furthermore, guanosine-5′-monophosphate (GMP) was reported to be 2.3-fold more active than IMP, while monosodium aspartate possesses only ~7% of the efficacy of MSG (Yamaguchi *et al.*, 1971). These purine-5′-nucleotides, occurring in many savory foods such as meat, fish, other seafood and mushrooms, are widely used as ingredients to enhance the flavor and mouth-feel of culinary products, snacks, soups, sauces and seasonings.

Very recently, molecular-biological investigations have succeeded in confirming the synergistic effect of purine-5′-ribonucleotides on the umami taste of MSG at the taste receptor level. Human T1R1/T1R3 heterodimeric receptors, made up by coexpression of the C-family G-protein-coupled receptor T1R1 and the related taste specific receptor T1R3, were demonstrated to respond to the umami-type taste stimulus L-glutamate (Li *et al.*, 2002). Although IMP and GMP, two hallmarks of taste enhancement, alone did not activate human T1R1/T1R3, these 5′-ribonucleotides strongly potentiated the L-glutamate-induced T1R1/T1R3

receptor response. In comparison, cytidine-5′-monophosphate, which does not enhance human umami-type taste perception, showed no effect on human T1R1/T1R3 receptors (Li *et al.*, 2002).

Besides the L-glutamate/purine-5′-ribonucleotide synergism, it has been reported that perception of the sweetness of sugars is increased in the presence of maltol or ethylmaltol (Bouchard *et al.*, 1968); for example, up to 15% of the sugar was substituted by adding 5-75 p.p.m. (based on sugar) of maltol without losing any intensity in sweetness. More recently, the cyclic enolones 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone, 2-hydroxy-3-methyl-2-cyclopenten-1-one and/or 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone were shown to enhance sweetness perception of sugars as well (Namiki and Nakamura, 1992). Solutions containing 5% sucrose and 15 parts (based on sugar) 2-hydroxy-3-methyl-2-cyclopenten-1-one tasted as sweet as an aqueous solution containing 20% sucrose. Although these cyclic enolones seem to intensify the sweet taste of sugars, their strong caramel- and seasoning-like odor might influence the overall aroma of foods.

Further examples of taste enhancement were reported for sodium chloride (NaCl). Sensory studies revealed several additives that permit reduction of the NaCl content in foods: basic amino acids such as L-lysine and L-arginine (Guerrero *et al.*, 1995); the peptide L-ornithyl-β-alanine (Tamura *et al.*, 1989*;* Seki *et al.*, 1990); and the disaccharide trehalose (Toshio *et al.*, 1997).

A survey of the literature showed that the sensory evaluation of potential taste-enhancing compounds had not, as yet focused on compounds which are not present in the foods *per se*, but are generated during food processing from precursors, e.g. by Maillard-type reactions from carbohydrates and amino acids; these compounds remain largely unknown. In order to bridge the gap between pure structural chemistry and human taste perception, a screening procedure called taste dilution analysis (Frank *et al.*, 2001; Ottinger *et al.*, 2001) was applied to high-pressure liquid chromatography (HPLC) fractions isolated from heated foods and heated carbohydrate/amino acid solutions of hexoses and L-alanine in order to locate sweetness enhancing compounds formed by food processing. This novel approach led to the discovery of the previously unreported *N*-(1-carboxyethyl)-6-hydroxymethyl-pyridinium-3-ol inner salt (see structure in Figure 1) in heated glucose/alanine solutions (Ottinger *et al.*, 2003). This so-called ‘alapyridaine’, which is naturally occurring in beef bouillon (unpublished data), does not show any taste on its own, but is able to enhance the sweetness of sugars, L-alanine and the artificial sweetener aspartame. Moreover, the (+)-(*S*)-configured compound was found to be the physiologically active enantiomer. Although this novel compound showed sweetness enhancing activity, enhancement of other taste qualities were not studied. The objectives of the present study were, therefore, to re-investigate the sweetness enhancement activity of alapyridaine and to investigate systematically the influence of (*R*/*S*)-, (+)-(*S*)- and (–)-(*R*)-alapyridaine on human umami, bitter, sour and salt perception using a selection of basic taste compounds.

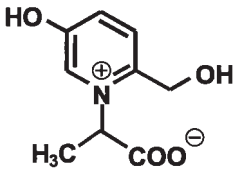

Figure 1 Structure of *N*-(1-carboxyethyl)-6-hydroxymethyl-pyridinium-3-ol inner salt (alapyridaine).

Materials and methods

Subjects

Twenty subjects with no history of known taste disorders completed this study (six women and 14 men, aged 25–38 years), all either employees or PhD students at the German Research Center for Food Chemistry in Garching, Germany. The assessors had participated earlier at regular intervals for at least 2 years in similar sensory experiments and were, therefore, familiar with the techniques and rating scales applied.

Stimuli

Stimuli representing the four classical basic tastes—sweetness, bitterness, sourness, and saltiness—as well as umami were included. Sucrose, L-phenylalanine, caffeine, citric acid, sodium chloride (NaCl) and MSG were obtained from Merck (Darmstadt, Germany); GMP and L-arginine were purchased from Sigma (Steinheim, Germany). Racemic *N*-(1-carboxyethyl)-6-hydroxymethyl-pyridinium-3-ol inner salt (alapyridaine) was prepared following the procedure recently reported in the literature (Ottinger *et al.*, 2003) and was purified using food-grade solvents. Both the enantiomers, (+)-(*S*)-alapyridaine and (–)-(*R*)-alapyridaine were stereospecifically synthesized and purified using food-grade solvents (Villard *et al.*, 2003). All alapyridaine samples were essentially free of sodium and chloride.

Procedures

The sensory panel was trained to evaluate the taste of aqueous solutions (4 ml each) of the following standard taste compounds by using a triangle test as described in the literature (Wieser and Belitz, 1975): sucrose (40 mmol/l) for sweet taste; citric acid (5 mmol/l) for sour taste; NaCl (12 mmol/l) for salty taste; caffeine (2 mmol/l) for bitter taste; and MSG (6 mmol/l, pH 6) for umami taste. All sensory analyses were performed in a sensory panel room at 22–25°C over three different sessions.

Influence of alapyridaine on the recognition threshold of basic taste compounds

Taste recognition thresholds, that is the concentrations at which the typical taste quality of a compound was just detectable, were determined in a triangle test using bottled water (Evian®) as the solvent. The pH value of the solutions was adjusted to 5.0, 7.0, or 9.0 by adding either aqueous hydrochloric acid (0.01 mmol/l) or aqueous sodium hydroxide solution (0.01 mmol/l). Serial 1:1 dilutions of the samples were presented in order of increasing concentrations to a trained panel of 20 persons in three different sessions, using the sip-and-spit method. At the start of the session and before each trial, the subject rinsed with water and expectorated. The samples, both blanks and taste solutions, were swirled around in the mouth briefly and expectorated. After indicating which glass vial showed the typical quality (umami, salty, sweet, sour, bitter) of the tastant, the participant received another set of two blanks and one taste sample. To prevent excessive fatigue, tasting began at a concentration level two steps below the threshold concentration that had been determined in a preliminary taste experiment. Whenever the panelist selected incorrectly, the next trial took place at the next higher concentration step. When the panelist selected correctly, the same concentration was presented again besides two blanks as a proof for the correctness of the data. The geometric mean of the last and the second-last concentrations was calculated and taken as the individual recognition threshold. The values between individuals and between separate sessions differed by not more than one dilution step.

Influence of alapyridaine on synergistic tastant pairs

To study the influence of alapyridaine on the taste synergism between MSG and GMP, aqueous solutions (pH 7.0) containing MSG, GMP and alapyridaine (0.6 mmol/l each) either alone, or in combination, were rated by the sensory panel on a scale from 0 (no taste detectable) to 3 (strong taste). The same experiment was performed for the synergism between NaCl and L-arginine using aqueous solutions of NaCl, L-arginine and alapyridaine (20 mmol/l each) either alone, or in binary combinations.

Relative taste-enhancing factor in iso-intense solutions

In order to determine how much the concentrations of sucrose, MSG and NaCl can be reduced in the presence of alapyridaine to meet the same taste intensity as aqueous solutions containing the basic tastants alone, aqueous solutions (pH 7.0) containing constant amounts of sucrose (20 mmol/l), MSG (3.0 mmol/l), or NaCl (20 mmol/l), but increasing concentrations of (*R*/*S*)-alapyridaine (0–30 mmol/l), were stepwise 1:1 diluted with water and the sensory panel was asked to evaluate the intensity of the sweetness, umami taste or saltiness of each dilution and to identify the dilution showing iso-intensity in taste with an aqueous reference solution containing sucrose (20 mmol/l), MSG (3.0 mmol/l), or NaCl (20 mmol/l). The relative taste-enhancing factor f_t of a mixture containing one of the basic tastants (sucrose, MSG, NaCl) and (*R*/*S*)-alapyridaine was related to a reference solution containing the corresponding basic tastant only and was determined as the ratio of the concentrations of iso-intensely tasting solutions of the basic tastant (c_t) and the binary mixture containing the basic tastant plus (*R*/*S*)-alapyridaine (c_{t+ala}), i.e.

$$f_{t,g}(c_t) = c_t/c_{t+ala}$$

Influence of alapyridaine on perception of tastant mixtures

To study the influence of alapyridaine on the taste intensities of binary tastant mixtures, the intensities of sweetness, umami and saltiness of aqueous solutions (pH 7.0) containing sucrose (12.5 mmol/l), MSG (1.5 mmol/l) and NaCl (10 mmol/l) in dual combinations and various amounts of (*R*/*S*)-alapyridaine (0, 1.5 and 7.5 mmol/l) were rated by the sensory panel on a scale from 0 (no taste detectable) to 3 (strong taste). The influence of (*R*/*S*)-alapyridaine on the taste intensities of a quaternary tastant mixture was studied with an aqueous solution (pH 7.0) of sucrose (12.5 mmol/l), MSG (1.5 mmol/l), NaCl (10 mmol/l) and caffeine (2.0 mmol/l) in the absence and presence of (*R*/*S*)-alapyridaine (1.5 and 7.5 mmol/l).

Results and discussion

Influence of alapyridaine on the recognition threshold of basic taste compounds

Determination of the recognition thresholds of aqueous solutions of MSG in the absence or presence of equimolar amounts of (*R*/*S*)-alapyridaine revealed that, independent of the pH value, the detection threshold of MSG (1.5 mmol/l) was significantly lower when (*R*/*S*)-alapyridaine was present; for example, the threshold decreased by a factor of four to 0.4 mmol/l (Table 1). Furthermore, we focused on the influence of the stereochemistry at the alanine moiety in alapyridaine on its taste-enhancing activity. At pH 7.0, (+)-(*S*)-alapyridaine was found to be twice as efficient as the racemic mixture, whereas the (–)-(*R*)-enantiomer did not affect the umami threshold of MSG at all (Table 1). The threshold concentration of an aqueous solution of the purine-5′-ribonucleotide GMP (0.3 mmol/l) was also decreased in the presence of equimolar amounts of (*R*/*S*)-alapyridaine, although the threshold only decreased by a factor of two (Table 1). A ternary solution containing MSG, GMP and (*R*/*S*)-alapyridaine in equimolar concentrations showed a taste threshold of 0.07 mmol/l, which is four-fold below the threshold of GMP and 6-fold below the threshold determined for a binary mixture of MSG and racemic alapyridaine (Table 1).

A second set of experiments was aimed at investigating the influence of alapyridaine on salt perception. The

Table 1 Influence of (*R/S*)-, (*R*)- and (*S*)-alapyridaine on the taste recognition thresholds of basic taste compounds

Taste sample	pH value	Taste quality	Threshold conc. [mmol/l water]	Threshold decrease
MSG	5.0/7.0	umami	1.5	–
+ (*R/S*)-alapyridaine	5.0	umami	0.4	4
+ (*R/S*)-alapyridaine	7.0	umami	0.4	4
+ (*R/S*)-alapyridaine	9.0	umami	0.4	4
+ (–)-(*R*)-alapyridaine	7.0	umami	1.5	–
+ (+)-(*S*)-alapyridaine	7.0	umami	0.2	8
GMP	7.0	umami	0.30	–
+ (*R/S*)-alapyridaine	7.0	umami	0.15	2
+ MSG + (*R/S*)-alapyridaine	7.0	umami	0.07	4
NaCl	7.0	salty	10.0	–
+ (*R/S*)-alapyridaine	7.0	salty	2.0	5
+ (–)-(*R*)-alapyridaine	7.0	salty	10.0	–
+ (+)-(*S*)-alapyridaine	7.0	salty	2.0	5
Sucrose	5.0/7.0	sweet	12.5	–
+ (*R/S*)-alapyridaine	5.0	sweet	6.0	2
+ (*R/S*)-alapyridaine	7.0	sweet	3.0	4
+ (*R/S*)-alapyridaine	9.0	sweet	1.5	8
+ (–)-(*R*)-alapyridaine	7.0	sweet	12.5	–
+ (+)-(*S*)-alapyridaine	7.0	sweet	1.5	8
Glucose	7.0	sweet	48.0	–
+ (*R/S*)-alapyridaine	7.0	sweet	3.0	16
+ (–)-(*R*)-alapyridaine	7.0	sweet	48.0	–
+ (+)-(*S*)-alapyridaine	7.0	sweet	1.5	32
L-phenylalanine	7.0	bitter	16.0	–
+ (*R/S*)-alapyridaine	7.0	bitter	16.0	–
Caffeine	7.0	bitter	0.5	–
+ (*R/S*)-alapyridaine	7.0	bitter	0.5	–
Citric acid	4.0	sour	1.25	–
+ (*R/S*)-alapyridaine	4.0	sour	1.25	–
+ (*R/S*)-alapyridaine	7.0	tasteless	–	–

detection threshold of an aqueous solution of NaCl was found to be significantly decreased in the presence of equimolar amounts of either racemic alapyridaine or the (+)-(*S*)-enantiomer; the threshold dropped by a factor of five from 10.0 to 2.0 mmol/l. On the other hand, the (-)-(*R*)-enantiomer did not show any influence on the taste threshold of NaCl (Table 1).

In order to confirm the recently observed effect of the novel compound alapyridaine on sweetness perception, we reinvestigated its influence on the threshold concentrations of the disaccharide sucrose and the monosaccharide glucose. Depending on the pH value, the detection threshold of sucrose (12.5 mmol/l), was significantly lower when alapyridaine was present; for example, the threshold decreased by a factor of four to 3.0 mmol/l at pH 7.0. Increasing the pH value to 9.0 led to a more pronounced effect—in fact, a threshold decrease by a factor of eight was determined (Table 1). On the other hand, decreasing the pH to 5.0 significantly lowered the activity of alapyridaine. Being well in line with the observations on the umami and salty taste, the enhancing effect was shown to be strongly dependent on the stereochemistry of the alanine moiety in alapyridaine. At pH 7.0, (+)-(*S*)-alapyridaine was found to be twice as efficient as the racemic mixture, with the thresholds of sucrose and glucose being decreased by factors of eight and 32, respectively (Table 1). As was seen for umami and salt tastes, the (–)-(*R*)-enantiomer did not affect the sweetness thresholds of either sugar.

Similar sensory studies on the role of alapyridaine in modifying bitter and sour taste perception revealed that neither the threshold concentrations of the bitter compounds L-phenylalanine and caffeine, nor that of the sour-tasting citric acid were influenced by the presence of alapyridaine (Table 1). In contrast to umami, sweet and salt taste, alapyridaine seems not to affect bitter and sour perception.

Taking all these data into consideration, it might be concluded that alapyridaine exhibits general taste-enhancing activities. Corroborating well its recently reported property of sweet-taste enhancement (Ottinger *et al.*, 2003),

equimolar amounts of alapyridaine were shown to significantly decrease the threshold concentrations of glucose and sucrose (Table 1). But, in addition, this compound also induced a significant threshold decrease for the umami taste of MSG and GMP solutions, as well as the salty taste of NaCl solutions. The taste perception of bitter compounds such as, caffeine, L-phenylalanine and naringine (data not shown), as well as the sour-tasting citric and lactic acids (data not shown) is, however, unaffected by alapyridaine. Studies on the role of the stereochemistry of alapyridaine revealed (+)-(*S*)-alapyridaine as the physiologically active enantiomer, whereas the (–)-(*R*)-configumer did not show any enhancing effect (Table 1).

Influence of alapyridaine on synergistic tastant pairs

Aimed at comparing the enhancing effect of L-arginine with that of alapyridaine, the salt intensities of various equimolar solutions containing NaCl, L-arginine and (*R*/*S*)-alapyridaine, either alone or in binary and ternary combinations, were determined (Figure 2A). The reference solution of the basic tastant NaCl was ranked with a score of 0.8, whereas solutions containing L-arginine, ala-

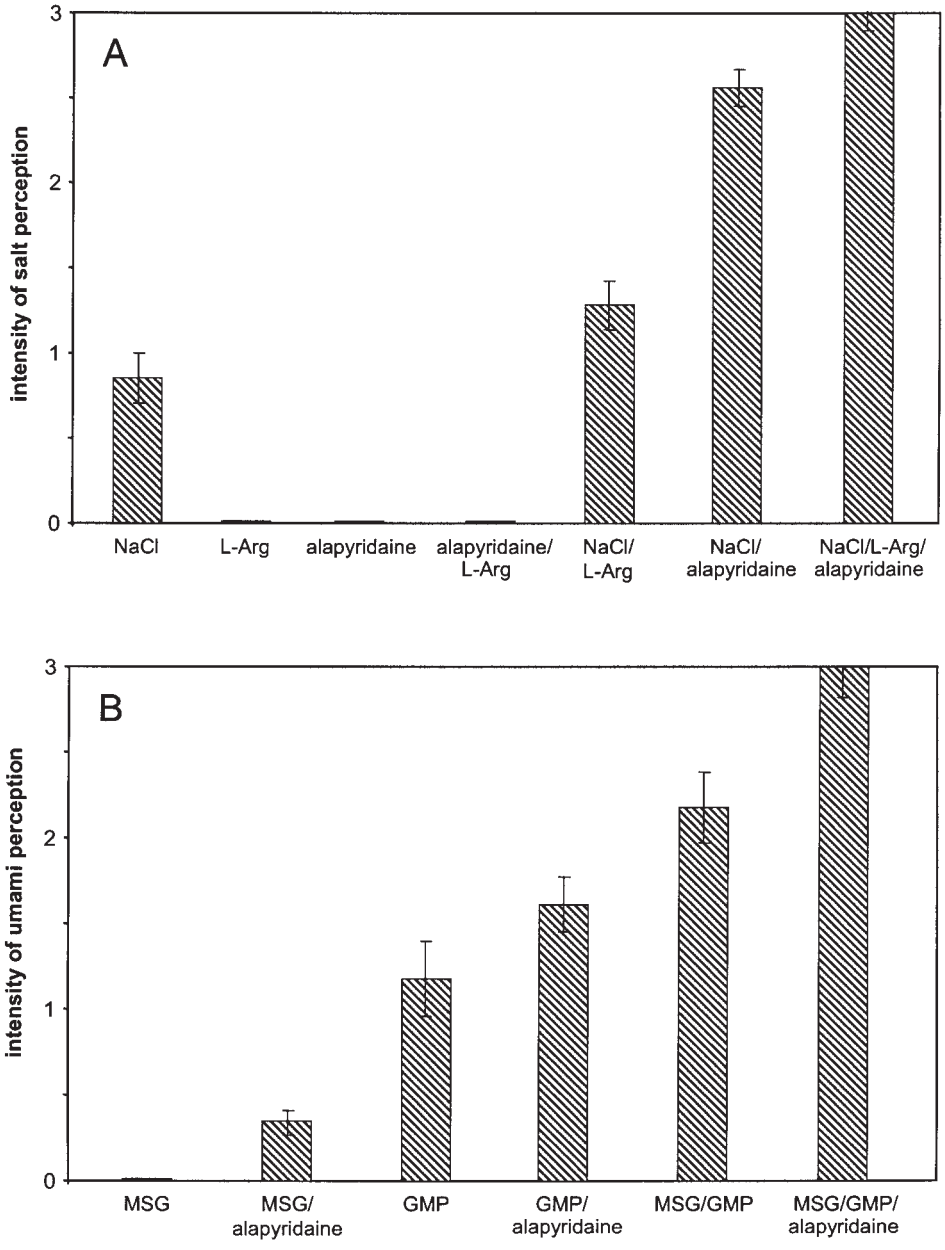

Figure 2 Influence of equimolar amounts of (*R*/*S*)-alapyridaine on the taste synergism between **(A)** NaCl and L-arginine (20 mmol each) and **(B)** MSG and GMP (0.6 mmol/l each). The error bars represent the standard deviation of the mean.

pyridaine, or a mixture of both, did not impart any salty taste impression (Figure 2A). In contrast, an equimolar mixture of NaCl and L-arginine was evaluated with a score of 1.3, thus confirming the salt-taste-enhancing effect of L-arginine reported in the literature (Guerrero *et al*., 1995). Substitution of L-arginine with alapyridaine, however, led to a significantly stronger effect: this solution was evaluated with an intensity of 2.6. The most intense salty taste, rated with a score of 3.0, was found for the ternary mixture of NaCl, L-arginine and alapyridaine (Figure 2A). These results clearly show that the enhancing effect of L-arginine on the salty taste of NaCl can be further intensified by alapyridaine.

In order to investigate the influence of alapyridaine on the enhancing effect of GMP on the umami taste of MSG, a comparative experiment was performed with GMP and MSG (Figure 2B). In the concentrations applied, the MSG did not impart any umami taste, whereas the GMP solution was evaluated with an intensity of 1.1 (Figure 2B). In the presence of equimolar amounts of alapyridaine, the intensities of both compounds increased; for example, the MSG and the GMP solutions were evaluated with scores of 0.4 and 1.6. In comparison, an equimolar MSG/GMP mixture was ranked with an intensity of 2.2, corroborating the well-known synergism reported for both umami compounds. Upon addition of alapyridaine, the umami intensity of the MSG/GMP mixture was further increased from a score of 2.2 to 3.0, thus demonstrating that the synergistic effect between MSG and GMP could be further intensified in the presence of alapyridaine (Figure 2B).

Taking all of these results into account, it can be concluded that the enhancing effect of L-arginine on the salty taste of NaCl (Guerrero *et al*., 1995), as well as that of GMP on the umami taste of MSG (Kuninaka, 1967; Yamaguchi, 1967; Yamaguchi *et al.*, 1971), can be significantly intensified by alapyridaine.

Relative taste-enhancing factor in iso-intense solutions

Iso-intensity experiments which were aimed at investigating how much the concentration of sucrose, MSG and NaCl can be reduced in the presence of alapyridaine to meet the same taste intensity as aqueous solutions containing the basic tastants alone, revealed that the relative sweetness of the mixtures containing sucrose and alapyridaine increases with increasing amounts of alapyridaine (Figure 3A). This shows again the enhancing potential of alapyridaine on the sweet taste of sucrose; for example, a solution containing 20 mmol/l sucrose and 20 mmol/l alapyridaine has to be diluted four-fold to match the sweetness of a solution containing 20 mmol/l sucrose only. In other words, a solution containing 5 mmol/l sucrose and 5 mmol/l alapyridaine is as sweet as a solution of 20 mmol/l sucrose (Figure 3A). Further increase of the alapyridaine concentration, however, revealed a slight reduction in taste-enhancing efficiency; for example, in the presence of 30 mmol/l alapyridaine, a relative sweetness factor of 3 was determined.

A corresponding experiment with an aqueous solution of MSG (3.0 mmol/l) revealed that the umami intensity of the mixtures containing MSG and alapyridaine increases with rising amounts of alapyridaine, up to a concentration of 6 mmol/l. This solution, containing 3.0 mmol/l MSG and 6.0 mmol/l alapyridaine, had to be diluted by a factor of four to match the umami intensity of a solution containing 3.0 mmol MSG only (Figure 3B). This means that a solution containing 0.75 mmol/kg MSG and 1.5 mmol/kg alapyridaine has the same umami intensity as the umami standard solution (3.0 mmol/l). As already determined for sucrose, a further increase of alapyridaine concentration led to a slightly lower effect (Figure 3B).

To gain information about how much the NaCl concentration can be reduced in the presence of alapyridaine, iso-solution experiments were performed with aqueous solutions of NaCl and alapyridaine. As outlined in Figure 3C, increasing the alapyridaine concentration increased the saltiness perception of the 20 mmol/l NaCl solution, running through a maximum at a concentration of 10 mmol/l alapyridaine and slightly decreasing again thereafter. The relative saltiness of 3.8 means that a solution containing 5.2 mmol/l NaCl and 10 mmol/l alapyridaine was evaluated with almost the same salt intensity as the 20 mmol/l standard solution.

Influence of alapyridaine on perception of tastant mixtures

The following experiments were aimed at investigating the effect of alapyridaine on the taste perception of binary mixtures of taste compounds. The intensities of the sweet and umami taste of a sucrose/MSG combination were evaluated with scores of ~1 when alapyridaine was absent (Figure 4A). The addition of 1.5 mmol/l alapyridaine induced a significant increase of both sweet (score 1.9) and umami (1.7) intensity (Figure 4). A further increase of the alapyridaine concentration to 7.5 mmol/l showed an even more pronounced effect: the sweetness was rated with the maximum score of 3.0 and the umami impression was evaluated with a score of 2.3.

A binary mixture containing NaCl (10 mmol/l) and MSG (1.5 mmol/l) was rated with a salty intensity of 1.0 and an umami intensity of 0.9 (Figure 4B). The addition of 1.5 mmol/l alapyridaine resulted in a strong intensity increase of both taste qualities; for example, the salty and umami notes were scored at 2.1 and 1.8, respectively. The presence of 7.5 mmol/l alapyridaine led to a further increase of the taste intensity, reaching a maximum score of 3.0 for the umami taste and a score of 2.8 for the salty note (Figure 4B).

The third combination consisting of sucrose (12.5 mmol/l each) and NaCl (10 mmol/l) was evaluated with nearly equal intensities for sweetness and saltiness, centering around 1.0 (Figure 4C). The presence of 1.5 mmol/l alapyridaine

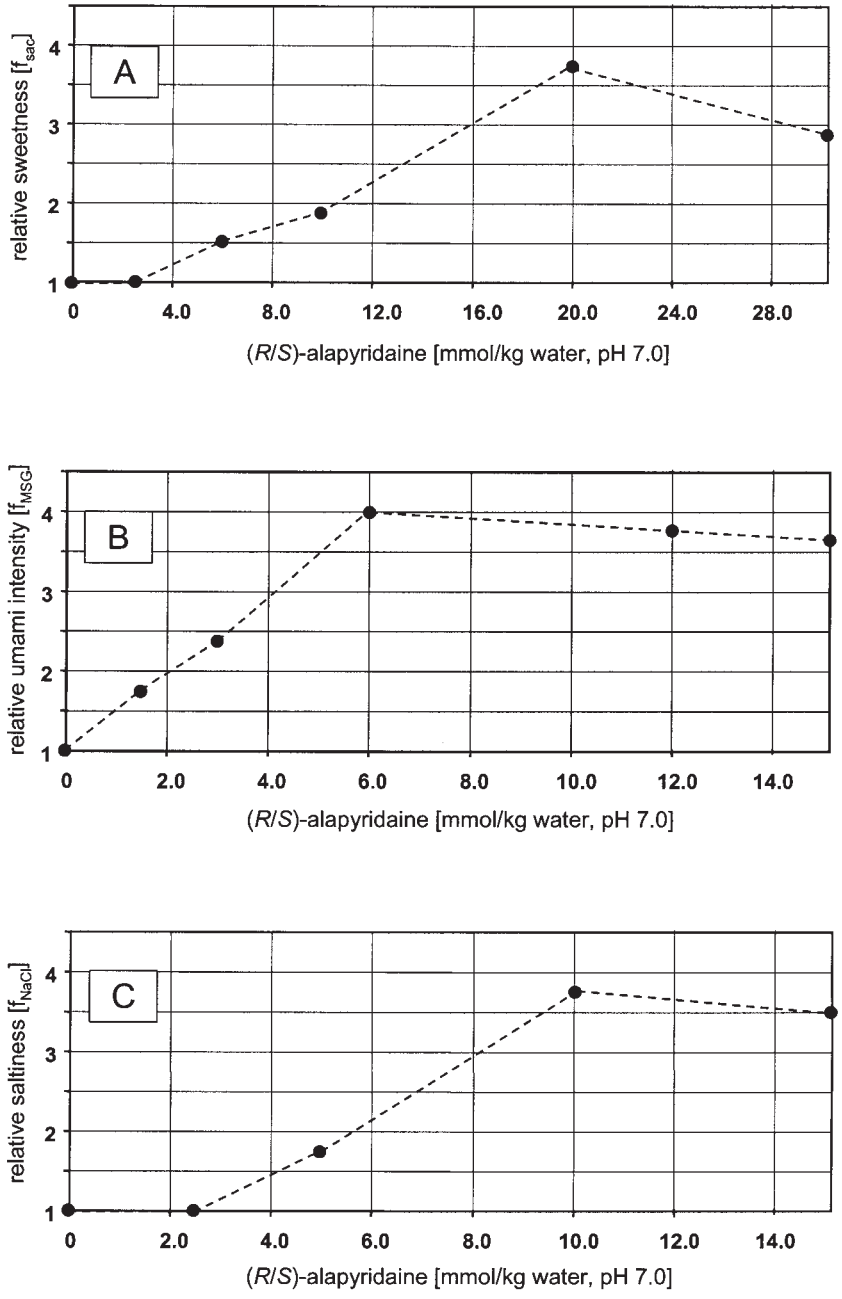

Figure 3 Relative taste-enhancing factor (*y*-axis) of (*R/S*)-alapyridaine in iso-intensely tasting solutions (pH 7.0) of **(A)** sucrose (20 mmol/l) and mixtures containing sucrose (20 mmol/l) and (*R/S*)-alapyridaine in various concentrations, **(B)** MSG (3.0 mmol/l) and mixtures containing MSG (3.0 mmol/l) and (*R/S*)-alapyridaine in various concentrations and **(C)** NaCl (20 mmol/l) and mixtures containing NaCl (20 mmol/l) and (*R/S*)-alapyridaine in various concentrations.

induced a significant increase of both sweet (1.5) and salt (1.7) intensity. However, in the presence of 7.5 mmol/l alapyridaine, the salty taste was more affected than the sweetness—the sensory panel evaluated the salty impression with a score of 2.8, whereas sweetness was only ranked at 2.0.

In a final experiment, the influence of alapyridaine on a quaternary tastant mixture containing sucrose (12.5 mmol/l), MSG (1.5 mmol/l), NaCl (10 mmol/l) and caffeine (2.0 mmol/l) was investigated (Figure 5). The taste intensities for the individual taste qualities were centered around a score of 1.0 when alapyridaine was absent. The sensorial evaluation of this quaternary solution in the presence of 1.5 mmol/l alapyridaine revealed a significant increase of the intensity of sweetness and umami taste, in particular and, to somewhat lesser extent, of saltiness, but the bitter

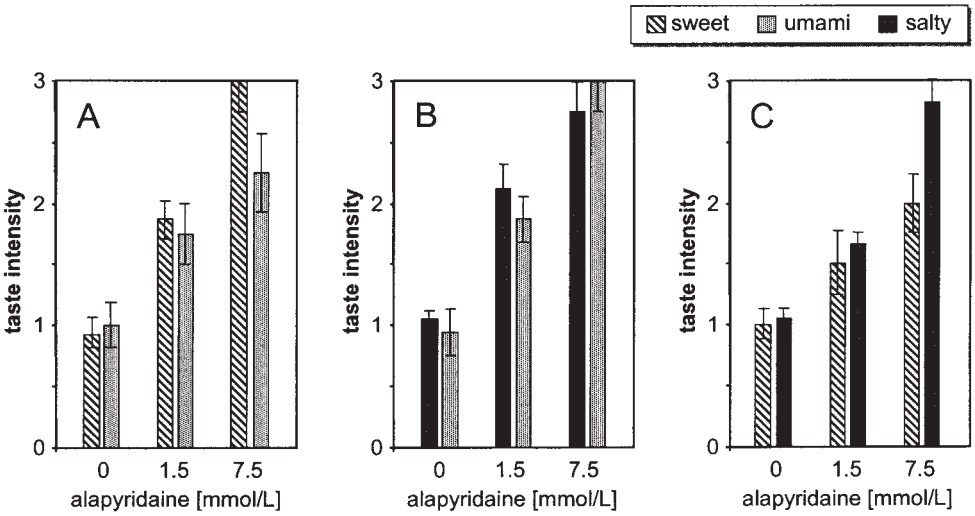

Figure 4 Influence of (*R/S*)-alapyridaine on the taste intensities of binary mixtures of taste compounds. **(A)** aqueous solutions (pH 7.0) of sucrose (12.5 mmol/l) and MSG (1.5 mmol/l) were presented in the absence or presence of alapyridaine. **(B)** Aqueous solutions (pH 7.0) of NaCl (10 mmol/l) and MSG (1.5 mmol/l) were presented in the absence or presence of alapyridaine. **(C)** Aqueous solutions (pH 7.0) of sucrose (12.5 mmol/l) and NaCl (10 mmol/l) were presented in the absence or presence of alapyridaine. The error bars represent the standard deviation of the mean.

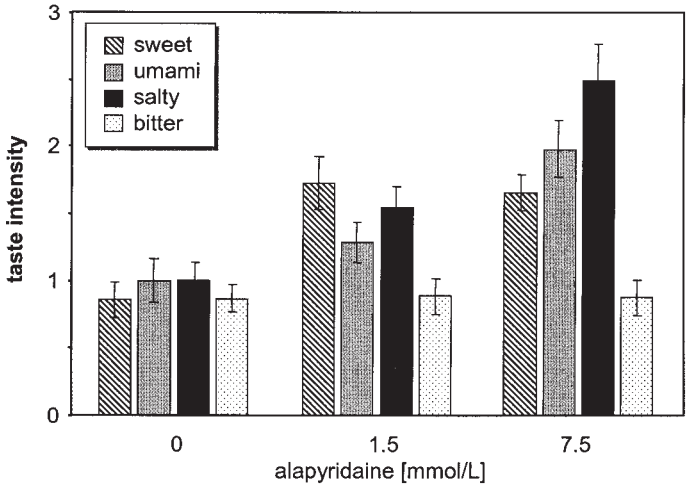

Figure 5 Influence of (*R/S*)-alapyridaine on the taste intensities of a quaternary tastant mixture. Aqueous solutions (pH 7.0) of sucrose (12.5 mmol/l), MSG (1.5 mmol/l), NaCl (10 mmol/l) and caffeine (2.0 mmol/l) were presented in the absence and presence of (*R/S*)-alapyridaine (1.5 and 7.5 mmol/l). The error bars represent the standard deviation of the mean.

taste was not affected. Addition of 7.5 mmol/l alapyridaine showed a more pronounced effect for the salty taste, which was rated with a score of 2.5. The umami taste was also further intensified to some extent, but no further enhancement of sweetness was detectable. Again, the bitter taste of caffeine was not affected.

Taking all these data into consideration, it might be concluded that (+)-(*S*)-alapyridaine enhances not only the taste of solutions containing single taste compounds, but also individual taste modalities of more complex tastant mixtures (Figures 4 and 5); for example; the umami, sweet and salty tastes of a solution containing MSG, sucrose, NaCl and caffeine was significantly modulated, thus illustrating that alapyridaine is a general taste enhancer.

Conclusions

Knowledge of the structure and physiological activity of taste-modulating compounds such as alapyridaine has major implications for the food industry, as well as for academic research. From the industrial point of view, the enhancing effect of (+)-(S)-alapyridaine on glucose and sucrose might be helpful for the development of low-calorie, sugar-reduced foods. The saltiness and umami enhancing properties of alapyridaine may prove important in the manufacture of low-sodium foods for patients with hypertension and might open new avenues for the production of umami-type-tasting savory foods with low L-glutamate contents.

As (+)-(*S*)-alapyridaine shows enhancing effects on both umami and sweet tastes, this compound might be helpful in the exploration of potential common mechanisms of receptor activation for sweetness and umami tastes. Very recent molecular-biological investigations have nicely demonstrated that human T1R1/T1R3 heterodimeric receptors respond to the umami taste stimulus L-glutamate, whereas T1R2/T1R3 dimers recognize diverse natural and synthetic sweeteners (Li *et al.*, 2002), thus implying that the T1Rs play a key role in umami as well as sweet taste and suggesting that sweet and umami taste receptors share a common unit. This common unit might be the clue to explain how (+)-(*S*)-alapyridaine is operating on a biomolecular level. To obtain a more comprehensive understanding of the fundamental mechanisms of taste

enhancement, (+)-(*S*)-alapyridaine might be a suitable compound to determine whether both the T1R1/T1R3 response to L-glutamate and the T1R2/T1R3 response to sweeteners can be potentiated in parallel.

The finding that alapyridaine also enhances salt perception indicates the existence of an additional way that this taste enhancer operates in taste cells. Whereas sweet and umami tastes are mediated by G-protein-coupled receptors, salt detection in the oral space is believed to be operated through independent mechanisms (Zhang *et al.*, 2003). As ion channels are commonly accepted as playing a key role in the transduction of Na^+ ions (Lindemann, 2001), such ion channels or downstream signaling elements might be targeted by (+)-(*S*)-alapyridaine. Because most of these molecules are not yet fully characterized, (+)-(*S*)-alapyridaine might be a suitable tool for systematic molecular-biological studies to increase knowledge of the molecular mechanisms of salt transduction in human taste cells.

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