REGULATION OF TASTE ACUITY BY THIOLS AND METAL IONS

By R. I. Henkin and D. F. Bradley

NATIONAL HEART INSTITUTE, BETHESDA, MARYLAND, AND
POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN, NEW YORK

Communicated by Melvin Calvin, October 25, 1968

Abstract and Summary.—The administration of thiol-containing drugs decreases taste acuity in man and animals. Copper (II) and zinc (II) administration returns taste acuity to normal levels. The results suggest that (1) thiols and metals are in dynamic equilibrium in the metabolic net, (2) regulation of taste acuity occurs through changes which thiols and/or metals bring about in the conformation of a protein which lines the pore of the taste receptor and its membrane, and (3) thiols normally play an inhibitory role in taste.

Recent clinical studies[1, 2] have shown that decreased taste acuity (hypogeusia) for each of four qualities of taste occurred in 32 per cent of the patients treated with D-penicillamine (β,β-dimethylcysteine) for scleroderma, cystinuria, rheumatoid arthritis, and idiopathic pulmonary fibrosis. These patients also had abnormally low, or lowered, serum copper and ceruloplasmin concentrations. Only 4 per cent of patients with Wilson's disease treated with D-penicillamine (D-pen) exhibited hypogeusia.[2] The drug reduced the high tissue copper content in patients with Wilson's disease, but not generally to normal. Such observations led to the hypothesis[2] that D-pen produced hypogeusia by lowering copper content. This hypothesis was tested[2] by administering cupric salts to patients with hypogeusia while continuing D-pen therapy. Taste sensitivity returned to normal in each patient treated with copper after the serum copper and ceruloplasmin concentrations returned to normal.[2]

These experiments in man were repeated in rats. Administration of D-penicillamine produced hypogeusia as indicated by elevated preference thresholds for salt and sugar. Administration of copper with continued administration of the drug returned taste acuity to control levels.[3]

In this report we demonstrate that thiols (RSH), as well as copper, play a role in the regulation of taste acuity.

Methods.—Taste acuity in man was evaluated by measurement of detection and recognition thresholds with a forced-choice, three-stimulus drop technique previously described.[4] Taste acuity in rats was calculated by measurement of preference thresholds determined with a two-bottle choice technique previously described.[5]

5-Mercaptopyridoxal (5-SH) was obtained from Merck Darmstadt, West Germany. D-pen was obtained from Merck, Sharp and Dohme as Cuprimine for experiments in man and, through the kindness of Dr. Elmer Alpert of Merck, Sharp and Dohme, for experiments in rats. Reagent grade $CuSO_4$ and $ZnCl_2$ were used. The determinations of copper and zinc in blood and urine were carried out by atomic absorption spectrophotometry with a Perkin-Elmer model 303 spectrophotometer.

Experiments and Observations.—A number of clinical observations suggested that thiols as well as copper were involved in the regulation of taste acuity. Initially, we were informed that during 1963, after treatment with 5-SH for six weeks, a total loss of taste (ageusia) had occurred in a rheumatoid-arthritis

patient (E. D.), although there was no associated alteration in serum copper concentration or urinary copper excretion.[6] Withdrawal of the drug was associated with a return of taste acuity to normal within two months.

Hypogeusia was also found in a patient with cystinosis, a disease associated with elevated disulfide (RSSR) levels.[7]

Some patients with rheumatoid arthritis have exhibited elevated serum concentrations of copper[8] and ceruloplasmin,[2] and usually an abnormally low serum RSH concentration.[9, 10] (These data suggest that an inverse relationship, which may be part of normal biochemical homeostasis, exists between copper and RSH concentrations in serum.) Those patients with rheumatoid arthritis who had elevated serum concentrations of copper and low serum concentrations of RSH after treatment with D-pen exhibited serum copper concentrations that were lower than normal[2] and increased serum RSH concentrations.[11] Thus, administration of D-pen adds thiols to the serum and removes copper from it. Under the conditions of lowered serum copper and increased serum RSH concentration, a number of these patients developed hypogeusia.[2] We performed experiments to document this inverse relationship and to determine whether the changes in taste acuity were directly related to changes in copper concentration, thiol concentration, or both.

Animal studies: Copper-RSH interrelationships were evaluated through studies of preference thresholds in rats fed D-pen or 5-SH. Four groups of male weanling Holtzman rats, individually caged, were fed a diet of ground Purina chow with 15 per cent dextrose added. The control group ate the diet alone; the second, the diet with 1 per cent D-pen by weight; the third, the diet with 1 per cent D-pen and $^1/_2$ per cent $CuSO_4$ by weight; the fourth, the diet with 1 per cent D-pen and $^1/_2$ per cent 5-SH. After taking their diets for 23 days, each rat was given a choice between water and 0.15 *M* or 0.30 *M* NaCl, and the preference was recorded.

The results are summarized in Table 1. Control rats showed the well-known preference for 0.15 *M* NaCl over water and rejection of 0.30 *M* NaCl. The rats fed D-pen showed an increased preference for both 0.15 *M* and 0.30 *M* NaCl. Rats fed 5-SH, not tested with 0.15 *M* NaCl, showed less rejection of 0.30 *M* NaCl than the controls. Thus, animals fed D-pen and 5-SH demonstrated one operational definition of hypogeusia. Rats fed both D-pen and

TABLE 1. *Taste preference and serum copper and zinc concentrations in normal rats and rats fed D-pen, D-pen plus copper (II), and 5-SH.*

Condition	No. animals	Per cent preference for 0.15 *M* NaCl vs. H_2O	Per cent preference for 0.3 *M* NaCl vs. H_2O	Total plasma Cu (μg/100 ml)	Total plasma Zn (μg/100 ml)
Controls	32	80 ± 1.0*	15 ± 4.0	93.8 ± 7.7	191.9 ± 14.1
Fed D-pen	32	99 ± 0.5	62 ± 6.0	10.1 ± 3.4	165.6 ± 10.4
Fed D-pen + Cu (II)	8	84 ± 1.5	20 ± 8.0	84.0 ± 1.8	173.0 ± 17.0
Fed 5-SH	8	—	33 ± 6.0	149.0 ± 13.7	201.7 ± 18.4

D-pen, D-penicillamine; 5-SH, 5-mercaptopyridoxal.
* Mean ± S.E.M.

copper (II) preferred 0.15 *M* NaCl to water and rejected 0.30 *M* NaCl, like the controls. Details of these experiments will be published elsewhere.[12]

The copper concentration in plasma of rats fed D-pen is about one tenth normal, but is not depressed in rats fed 5-SH, although both groups showed either less rejection of or actual preference for 0.30 *M* NaCl (Table 1).

D-pen administration affects metabolism of zinc (II), but plasma zinc concentrations did not differ among the four groups of rats (Table 1). However, there may be changes in other body compartments not in dynamic equilibrium with plasma. Treatment of patients with D-pen causes no change or an increase in plasma zinc concentration, an increase in urinary zinc excretion,[13, 14] and an increase in tissue zinc, due to an increase in its gastrointestinal absorption.[14] By contrast, whole-body copper content is depleted, serum copper concentration falls, and excretion of urinary copper rises. Patients with Wilson's disease have elevated tissue concentrations and low serum concentrations of copper. Treatment of these patients with D-pen lowers tissue and serum copper and increases urinary excretion of copper.

Clinical studies: At the National Cancer Institute, a patient (G. B.) with multiple myeloma of the rare G_3 subclass[15, 16] spontaneously reported an impairment of taste. Although most patients with myeloma have normal taste acuity,[17] she stated that all food was tasteless and that only after adding of excessive amounts of salt or sugar to her food did she obtain any sensation of salt or sweet. Both the patient and her nurses noted that she tolerated without complaint various noxious-tasting medicines which other patients refused or took with reluctance. Detection and recognition thresholds for the four taste qualities were significantly elevated. Serum copper concentration was within normal limits and urinary copper excretion was elevated (Table 2).

In previous examples of hypogeusia, serum concentrations of thiols were increased, serum concentrations of copper were decreased, or both. This patient had normal copper levels; therefore we postulated that her hypogeusia was related to an elevated level of thiols. G-myeloma proteins contain approximately 20 inter- and intrachain disulfide bonds, and the high concentration of this protein in the patient's serum might be a source of exchangeable RSH or RSSR. Thus, the patient might have an excess of RSH or RSSR resulting from her disease, as was the case with the patient with cystinosis.

We attempted to correct this patient's hypogeusia by administering copper (II) to remove her exchangeable RSH or RSSR by chelation or complex formation. Daily oral administration of 20 mg of copper as $CuSO_4$ returned her taste acuity to normal for the four taste qualities within four days (Table 2). Concomitantly, her serum copper concentration increased. During an additional four days of copper administration, her taste acuity remained normal while her serum copper concentration continued to increase. During treatment the quantity of salt and sugar that she used decreased, and revulsion to a variety of medicines, including $CuSO_4$, appeared. After copper therapy was stopped, hypogeusia appeared within 48 hours. She again used excessive amounts of salt and sugar and again took medicines without complaint.

We next investigated the question whether other metals in the group of transi-

tion metals which might chelate or complex with this patient's postulated elevated levels of RSH or RSSR could also affect her taste acuity. Within four days after daily oral administration of 60 mg of zinc as $ZnCl_2$, taste acuity for all taste qualities was normal. This change was associated with an increase in her serum zinc concentration to normal. Continuation of zinc therapy maintained her taste acuity and serum zinc concentrations at normal levels. However, 24 hours after zinc therapy was discontinued, hypogeusia for each of the four taste qualities was observed.

Discussion.—These experiments and observations show that taste acuity can, in certain instances, be altered in a predictable direction through the administration of thiol drugs or copper and zinc salts. Administration of thiol drugs lowers taste acuity. Administration of copper (II) during thiol drug therapy increases taste acuity. Administration of copper (II) or zinc (II) to a patient with hypogeusia accompanying onset of multiple myeloma increased taste acuity. Recent experiments have also shown that hypogeusia can be produced by raising an animal on a copper-free diet.[7]

Control of taste acuity is undoubtedly a highly complex biological process, but we wish to propose a simple chemical model of that part of the process related to the above observations. Since taste acuity decreased with thiol administration, a model in which thiols are given a primary role in the control of taste acuity assumes that thiols are normally inhibitory in the taste process. We therefore propose that thiols act as inhibitors of taste acuity by engaging in sulfhydryl-disulfide interchange[18] equilibria with a disulfide-containing protein (R′SSR″) involved in one of the chemical-level steps of gustation; for example,

$$\mathrm{RSH} + \underbrace{\mathrm{R'SSR''}} \rightleftharpoons \mathrm{RSSR'} + \underbrace{\mathrm{R''SH}}, \quad (1a)$$

TABLE 2. *Taste thresholds and serum and urinary copper and zinc concentrations in a patient with multiple myeloma.*

Condition	Taste Thresholds (mM/liter)				Serum (μg/100 ml)		Urine (μg/24 hr)	
	NaCl	Sucrose	HCl	Urea	Total copper	Total zinc	Total copper	Total zinc
Untreated	300/300*	300/300	30/300	1000/1000	95	42	200	400
Copper administration (4th day)	60/60	60/60	6/6	150/150	123	56	97	468
Copper administration (8th day)	12/30	12/30	0.8/3	90/120	170	54	19	432
First day after stopping copper administration	150/150	150/150	60/60	300/300	187	85	20	403
Third day after stopping copper administration	800/800	800/800	60/60	1000/1000	168	90	170	323
Zinc administration (4th day)	60/60	12/12	3/3	150/150	118	73	140	265
First day after stopping zinc administration	150/150	60/60	6/30	300/500	105	71	169	240
Normal median	12/30	12/30	0.8/3	120/120				
Normal mean ± S.E.M.					105 ± 4	96 ± 4	53 ± 12	140 ± 15

* Numerator of fraction is *detection threshold.* Denominator of fraction is *recognition threshold.*

or

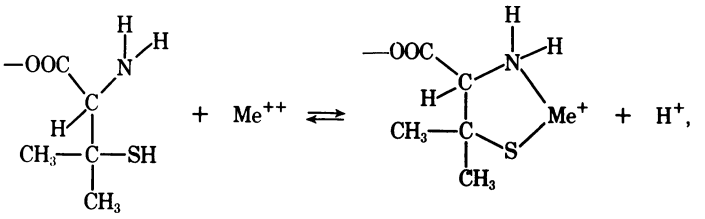
$$\mathrm{RSSR} + \underbrace{\mathrm{R'SSR''}} \rightleftharpoons \mathrm{RSSR'}\ \underbrace{\mathrm{R''SSR}}. \qquad (1b)$$

If the protein were catalytically active, this reaction could completely inhibit the activity of individual protein molecules. If the protein were not catalytically active but lined the holes of a membrane, the reaction could inhibit taste by partially unfolding the protein, increasing its hydrodynamic radius of gyration, and blocking the holes.

The fraction of enzyme molecules inhibited or of holes blocked would be determined by the local thiol concentration and would increase or decrease reversibly[19] with the free thiol concentration at the site where the protein is located. As the thiol concentration increased, detection and recognition thresholds would rise as higher tastant concentrations would be required to produce the taste sensation. (A tastant is a substance that can be detected and recognized by its taste quality.)

Reaction (1) is so general that we might expect thiol drugs to affect many metabolic reactions in addition to taste acuity. Treatment with D-pen or 5-SH does produce metabolic alterations in many organ systems. Nevertheless, of the senses, only taste appears to be significantly affected and each of the four qualities of taste is impaired. We feel that this surprising result is an important clue to formulation of a chemical-level description of taste.

In order to account for the reversal of thiol inhibition by copper and zinc, we further propose that thiols and metal ions are in near-chemical equilibrium with one another in the body through a group of reactions[20–22] including chelation,



(2)

complex formation,

$$2\mathrm{RSH} + \mathrm{Me^{++}} \rightleftarrows (\mathrm{RS})_2\mathrm{Me} + 2\mathrm{H^+}, \qquad (3)$$

or oxidation-reduction,

$$2\mathrm{RSH} + \mathrm{Cu\ (II)} \rightleftarrows 1/2\ \mathrm{RSSR} + \mathrm{RSCu(I)} + 2\mathrm{H^+}. \qquad (4)$$

In a biological steady state, local thiol, disulfide, and metal ion concentrations will be determined by the simultaneous satisfaction of these multiple equilibria (1)–(4) plus other simultaneous equilibria which might be operative in the system. If the level of $\mathrm{Me^{++}}$ were increased to a higher than normal stationary state value, we would expect the equilibria of reactions (2)–(4) to be shifted to the right so that (RSH) is decreased. This would shift the equilibria of reaction (1) to the left, causing a refolding of the protein R'SSR'' and a concomitant increase in taste acuity. It is, of course, the change in free metal

ion concentration and not the total metal ion concentration that determines the direction in which these equilibria are shifted.

These reactions, which have thiols as a common reactant, describe how metal ions, though not directly involved in any chemical step in taste, could nevertheless affect taste acuity. This chemical-level description of taste acuity accounts for the observation reported herein that Cu(II) administration in the face of persistently high thiol administration returned taste acuity to normal.

Kinetic reversibility of these chemical reactions is reflected in return of hypogeusia within 48 hours after metal ion administration is stopped.

The extent to which the equilibria are shifted by a given change in metal ion concentration will be determined by the equilibrium constants for the reactions involved. The smaller the equilibrium constants, the smaller will be the change in thiol concentration accompanying any given change in metal concentration. However, to remove a given number of equivalents of free RSH, at least as many equivalents of metal ion must be added to the system. In general, the equilibrium constants for reactions (2) and (3) are large[20, 22] but vary markedly with the metal and thiol involved. Copper (II) and zinc (II) form about equally stable mercaptides with the RSH of bovine serum albumin.[21]

An alternative explanation is that metals are activators of a step in the regulation of taste acuity and that thiols inhibit indirectly through control of metal ion concentration by means of reactions (2)–(4). The metal could be a cofactor of an enzyme; for example, as zinc in carbonic anhydrase[23] and carboxypeptidase A[24] and as copper in mushroom tyrosinase.[23] Alternatively, it could activate the step by binding the protein into a larger aggregate.[18] The aggregate might be active catalytically or fit into a specific site in the taste apparatus. Although both copper and zinc could bind to the same enzyme, it is unlikely that they would both activate it.[24] To account for the activity of both copper and zinc in returning taste acuity to normal, we suppose that one activates the enzyme; the other acts by reducing free thiol concentrations through reactions (2)–(4).

A third possible explanation is that thiols and one of the metals act together in the activity of a protein; for example, as zinc and cysteine in carboxypeptidase A[26] and carbonic anhydrase.[23] Recent high-resolution X-ray diffraction studies have shown that Zn (II) and cysteine are not in contact in either carboxypeptidase[26] or carbonic anhydrase,[23] although they act together in the activity of the enzyme.

The X-ray studies on the former enzyme suggest a simple mechanism by which part of the taste process might occur. When the substrate binds to carboxypeptidase there is a conformational change in the protein involving the movement of a tyrosyl hydroxl 14 Å toward the substrate. The binding of a tastant to a metal and thiol-containing protein could produce a conformational shift in the protein of comparable magnitude. If the protein lined a hole of a membrane through which chemical species must pass by diffusion, this conformational change could affect the pore diameter and effective membrane permeability. Subsequent random dissociation of the tastant from the binding site would return the protein to its original shape and the pore to its original diameter. Local

concentrations of other thiols could be inhibitory by competing for or with the normal metal ligands, one of which could be the SH of a cysteine residue of the protein, and prevent tastant binding.

Regardless of how we distribute the roles of thiols and metals, we arrive at chemical descriptions of taste which have certain features in common. These models, along with some recent observations of taste buds obtained with the scanning electron microscope,[27] have encouraged us to propose an anatomical-physiological description of the regulation of taste acuity. This model is comprised of two major events, a preneural or peripheral event and a neural event, each of which is comprised of many chemical events. The taste bud has been shown to consist of a large pore and a membrane in a surrounding papilla. In the preneural event the chemical to be tasted, i.e., the tastant, enters the pore and diffuses down its length or through its lateral surfaces. In the neural event the tastant directly or indirectly depolarizes gustatory nerve fibers.[28-30] We suppose that conformational changes of the protein molecules lining the pore and the membrane of its lateral surfaces, regulated by the multiple equilibria involving thiols and metals described above, in turn control the diameter and permeability of the pore and its membrane. We designate the protein molecules which regulate the amount of tastant that passes through the taste bud pore per unit time and through smaller pores of its membrane as the gate-keeper protein. To change the effective diameter of small pores of the membrane we need invoke only small conformational changes such as could be produced by tastant or substrate binding. Binding of a substrate to a protein has been observed to produce conformational changes of the order of magnitude of 10 Å in several proteins.[24, 26] To make an appreciable change in the effective diameter of the larger taste bud pore would require a more drastic conformational change such as produced by protein aggregation or unfolding.

If the gate-keeper protein reduces the pore diameter and membrane permeability to a value smaller than normal following administration of a thiol-containing drug, detection thresholds would be elevated because a greater concentration of tastant would be needed to raise the net flux through the pore and its membrane to a level which would initiate the neural events. If metals such as copper or zinc were administered concomitantly, the gate-keeper protein would return toward its normal conformation, increase the pore size and membrane permeability, and reduce the concentration of tastant required to trigger the neural events. Conformational changes of the gate-keeper protein would produce parallel changes in threshold for each of the four qualities of taste, as observed. Carbohydrate-active steroids are also inhibitors of taste acuity.[4] Removal of carbohydrate-active steroids increases taste acuity above normal by a factor of 10^3.[4] Coincident with this there is an increase in velocity of transmission of neural impulses along axons[31] and a decrease of velocity across synapses.[32] Neural excitability is also significantly increased.[33] Replacement of carbohydrate-active steroids returns taste acuity and neural function to normal within a relatively short time. Thus the normal role of carbohydrate-active steroids in the regulation of taste acuity in man is one of strong inhibition of neural events.

We propose that the normal role of thiols in the regulation of taste acuity in man is also one of strong inhibition, but of preneural events.

The authors wish to thank Drs. Timothy Canty, William Terry, Morley Kare, and Harry Keiser, and Miss Silja Meret for their kind assistance throughout these studies.

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