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Impaired inositol trisphosphate generation in carbachol-stimulated submandibular gland acinar cells from ascorbate deficient guinea pigs

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Ascorbic acid (AA) is a ubiquitous component of animal tissues which is sequestered by cells achieving very high concentrations not only in the neurohormonal secreting cells of the hypothalamus and in endocrine cells of the pituitary and adrenal glands but also in the acinar cells of salivary glands. The ascorbate-dependent processes occurring in salivary glands are not clear. We hypothesized that AA might have some physiological role in the secretory processes of neurotransmitters in salivary glands. Using a guinea pig model of marginal ascorbate deficiency not complicated by anorexia with its attendant problems of reduced intakes of energy, protein, and other essential nutrients, we examined the specific role of vitamin C in stimulated salivary gland exocytosis in vitro. Ascorbate deficiency elicited prominent reductions in stimulated whole saliva flow rate (35.0 ± 6.0 compared with 80.0 ± 8.1 μL/min in control; $P < 0.025$). The ascorbate concentration in the deficient gland was only 4.4% of the control level ($P < 0.002$). Muscarinic-cholinergic receptor stimulation of dispersed submandibular gland acinar cells with carbachol (20 μM) yielded a mean IP_3 generation (percent of unstimulated sample) of 236 ± 32 in the control animals, which was significantly ($P < 0.025$) higher than the mean of 120 ± 11 for the deficient guinea pigs. In ascorbate-deficient guinea pigs refed an adequate vitamin C diet for 2 to 7 days (mean of 5 days), mean stimulated IP_3 generation (188%) showed marked recovery. An equally prominent impairment in $[Ca^{2+}]i$ elevation was associated with reduced IP_3 production in ascorbate deficiency (292 ± 31 nM in deficient animals versus 578 ± 36 for the control group). The resting level of $[Ca^{2+}]i$ was nonsignificantly higher in the control than in the malnourished group. Our data supported the conclusion that impairment in the muscarinic-cholinergic transmembrane signaling cascade system, particularly the metabolism of phosphatidylinositol phosphates, could explain the hyposalivation in vitamin C–deficient guinea pigs. The difficulty of extrapolating findings from experimental animals to humans notwithstanding, it should be noted that most of the seemingly unrelated factors/conditions implicated in the genesis of xerostomia (dry mouth) in the human promote increased requirement for and/or tissue depletion of vitamin C. (J. Nutr. Biochem. 6: 557–563, 1995.)

Keywords: secretion; ascorbate status; phosphatidylinositol 4,5-biphosphate turnover

Introduction

Salivary glands possess a variety of cell-surface receptors which are stimulated by the autonomic nervous system to promote secretion of saliva.[1–3] Norepinephrine (sympathetic) and acetylcholine (parasympathetic), which stimulate the adrenergic (α and β) and muscarinic-cholinergic (mAChR) receptors, respectively, are the key neurotransmitters involved.[2,3] These receptors, when stimulated, couple through G proteins (Guanine nucleotide-binding regulatory proteins) to initiate intracellular signaling events which promote generation of specific messenger molecules (cations, nucleotides, phospholipid metabolites).[1,3] Stimulation of the β-adrenergic receptors elicits protein secretion primarily, while α-adrenergic and mAChR stimulation results mainly in fluid secretion,[1] although there is increasing

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evidence of interactions or "cross-talk" between the various signaling systems.[2,4,5] Stimulation of mAChR activates inositol phospholipid-specific phospholipase C (PLC) which catalyzes hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) to generate diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3).[6] The IP_3 induces release of Ca^{2+} from internal stores.[1,6]

In an earlier study we reported prominently reduced stimulated whole saliva flow rate in ascorbate deficient guinea pigs,[7] a finding consistent with observations of xerostomia and hypofunction of salivary glands in scorbutic humans.[8] The mechanistic reason for hyposalivation in ascorbate deficiency was not clear. High concentrations of intracellular ascorbic acid (AA) are found in neurohormonal secretory cells of the hypothalamus, pituitary, and adrenal glands[9–11] as well as in exocrine acinar cells of the parotid gland,[12,13] and there is increasing evidence that AA affects secretory processes of neurotransmitters/neurohormones.[10,11] Ascorbic acid modulates cAMP levels[14] and several aspects of tissue metabolism of $[Ca^{2+}]i$.[15] Additionally, ascorbate, probably through its role in recycling vitamin E (α-tocopherol) radical to the active antioxidant form, prevents oxidative damage to cell membranes induced by aqueous radicals.[16]

Since receptors, G proteins, or effectors are influenced by many factors including the nutritional status of the host,[17,18] this study was designed to examine the impact of dietary ascorbic acid deficiency on peak IP_3 generation and cytosolic $[Ca^{2+}]i$ levels in the submandibular gland acinar cells of male guinea pigs stimulated with carbachol, a muscarinic-cholinergic Ca^{2+} mobilizing agonist. The guinea pig, like the primates, is dependent on dietary sources for ascorbic acid, and the submandibular gland of this species, with its rich supply of adrenergic and cholinergic nerves,[19] is characterized predominantly by serous acini.[20]

Methods and materials

This study was approved by the Institutional Animal Care and Use Committee, University of Maryland, Baltimore, MD.

Materials

Male Hartley guinea pigs (Charles River Laboratories, Inc., Wilmington, MA USA) aged about 21 days, and weighing 300 to 350 g were purchased for the study. The three isocaloric semisynthetic diets (adequate, totally devoid of vitamin C, and 50 ppm vitamin C) were prepared for use in pelleted form by United States Biochemicals (Cleveland, OH USA). The detailed compositions of the diets are shown in *Table 1*. The diets were stored in a refrigerated room (0°C), and random batches were analyzed periodically to validate the vitamin C content. The study involved use of carbachol, bovine testicular hyaluronidase (types 1-S, 270 U/mg), Quin-2-AM, and EGTA (Sigma Chemical Co., St. Louis, MO USA). The incubation medium used was a modified Hanks' balanced salt solution containing 137 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4$, 1.28 mM $CaCl_2$, 0.33 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 5.6 mM glucose, 33 mM HEPES (pH 7.4), and 0.1% bovine serum albumin (BSA) as described by Baum and colleagues.[21] The dispersion medium was made up of the incubation medium containing 100 U/mL of collagenase (type CLSPA; Worthington Biochemical Corp., Freehold, NJ USA) and 0.1 to 0.2 mg/mL of hyaluronidase (type 1-S; Sigma Chemical Co.).[21] myo-2[^{3}H]-Inositol was from DuPont NEN Research Products, Boston, MA, USA, Opti-fluor scintillation fluid was purchased from Packard Instrument Co., Meriden, CT, USA, and the A61X-8 resin (formate form, 200–400 mesh) was a product of BioRad, Richmond, CA, USA. All other reagents used were of the highest available grade.

Table 1 Composition of semisynthetic diets*

Ingredients per kg diet	Control diet	Ascorbate deficient diet	Marginal ascorbate diet
Casein (vitamin free) (g)	300	300	300
Corn oil (g)	73	73	73
Sucrose (g)	103	103	103
Celufil (g)	150	150	150
Corn starch (g)	200	202	202
Dextrose, anhydrous (g)	78	78	78
Potassium acetate (g)	25	25	25
Magnesium oxide (g)	5	5	5
Salt mixture, Briggs A (g)	60	60	60
Choline chloride (g)	2	2	2
Inositol (g)	2	2	2
Ascorbic acid (g)	2	0	0.05

*Each diet contained (mg/kg) thiamine hydrochloride (16.0), riboflavin (16.0), pyridoxine hydrochloride (16.0), calcium pantothenate (40.0), niacin (200.0), biotin (0.6), folic acid (10.0), vitamin B_{12} (0.04), vitamin A acetate (6.0), vitamin D_3 (0.04), dl-alpha-tocopherol acetate (20.0), and vitamin K_3, oil soluble (2.0). Additionally, sodium propionate (0.5%), a mold inhibitor, and deionized water (4.0%) were added before pelleting.

Induction of ascorbate deficiency

All the guinea pigs were housed individually in plastic cages in a room maintained on a fixed photoperiod of 12-hr light/dark cycle and allowed free access to drinking water. During the first week in our animal facilities, the guinea pigs were fed ad libitum with an adequate vitamin C containing semisynthetic Reid-Briggs diet (United States Biochemicals/Amersham Life Science, Cleveland, OH USA). The animals that failed to show an appropriate gain in body weight during the 1 week period of quarantine were eliminated from the study. The rest were randomized into two major groups, namely Group A (control) and Group B (vitamin C–deficient).

Group A continued on the control diet (*Table 1*). Group B animals were initially fed ad libitum with a diet totally devoid of vitamin C for 12 days to desaturate the tissues of this nutrient and then transferred to a diet supplying a maintenance dose of ascorbic acid (50 ppm of the diet) for 3 to 5 weeks (*Table 1*). All the animals were allowed free access to drinking tap water. Our model of ascorbate-deficient guinea pigs had earlier been shown to have food consumption pattern and weight curves comparable to the controls and lacked the hemorrhages characteristic of acute scurvy in this species.[7,22,23]

Sample collection and assays

Sample collection was routinely carried out between 0900 and 1000 hr following an overnight fast. Anesthesia was induced by intramuscular injection of a cocktail which supplied (per 100 g of body weight) 6.7 to 10.0 mg of ketamine HCl (Sigma), 1.0 mg of xylazine HCl (Sigma), and 0.15 mg of butorphanol tartrate (Aveco Company, Fort Dodge, IA USA). Stimulated whole saliva was

collected from some guinea pigs as previously described,[7] excluding the guinea pigs used for IP_3 and $[Ca^{2+}]i$ evaluation. Saliva secretion was induced with intraperitoneal injection of a mixture supplying 5 mg/kg of body weight of isoproterenol hydrochloride (Sigma) and 4 mg/kg of body weight of pilocarpine hydrochloride (Sigma). Cells from finely minced submandibular gland tissue were obtained by enzymatic digestion as described by Baum et al.[5,21]

Measurement of IP_3 generation was as previously described by others[21,23] with the unstimulated contralateral gland from the same guinea pig serving as a control for the stimulated gland. In effect each animal served as its own control. Right and left submandibular salivary glands were removed from the animal and were finely minced on a McIlwain tissue chopper. The minced glands were separately incubated in 2.5 mL of Hanks' balanced salt solution buffered with HEPES to pH 7.5 (HBSS-H) containing 0.01% BSA, 100 U/mL of collagenase, and 0.2 mg/mL of hyaluronidase. The incubation was performed in a metabolic shaker (150 rpm) at 37°C for 60 min. The tissue was dispersed and gassed with CO_2 every 20 min. After the incubation the resulting suspensions were washed 3 times in HBSS-H by centrifugation at 3,500 rpm for 10 sec. The cells were then resuspended in 2 mL of HBSS-H containing 40 μCi/mL of myo[^{3}H] inositol (specific activity: 23.45 Ci/mMol) and incubated at 37°C for 80 min with gassing every 20 min. Thereafter, cells were washed 3 times with HBSS-H without BSA and allowed to recover at 37°C for 10 min. LiCl (10 mM) was added 10 min prior to the addition of any agonist. Carbachol (2×10^{-5} M) was added to one of the gland preparations while an equal volume of HBSS-H was added to the other to serve as a control for measurement of basal levels of myo-inositol phosphates (IPS). Preliminary experiments showed that in our system, addition of 2×10^{-5} M carbachol produced maximum secretion of amylase and peroxidase as well as maximal rise in $[Ca^{2+}]i$ and IP_3 generation with no significant changes in these parameters when carbachol level was increased to 10^{-4} M. The reactions were stopped after 45 sec with the addition of ice-cold 100% TCA to yield a 10% (v/v) solution which was kept on ice for 30 min. The resulting precipitate was removed by centrifugation, and excess trichloroacetic acid (TCA) was removed by ether extraction (4 times). The sample was then adjusted to pH 7.0 by the addition of 0.1 N NaOH. Each sample was then applied to a column (0.8 × 4 cm) containing 2 mL of Bio-Rad AG 1×-8 resin (formate form, 200–400 mesh). The IPS were eluted by stepwise addition of H_2O (18 mL), 5 mM disodium tetraborate/60 mM sodium formate (8 mL), 0.1 M formic acid/0.2 M ammonium formate (12 mL), 0.1 M formic acid/0.4 M ammonium formate (16 mL) and 0.1 M formic acid/1.0 M ammonium formate (12 mL). Fractions (0.5 mL) were collected and added to 5 mL of Opti-Fluor for counting of radioactivity (Liquid Scintillation Counter, LS5801, Beckman Instruments, Nuclear Systems Operations, Fullerton, CA USA). The inositol phosphates were identified according to Berridge et al.[24] and values reported as a percent of the unstimulated preparation from the same animal.

Measurement of cytosolic Ca^{2+} was as described by Baum et al.[21] using Quin 2-AM fluorescence. The cell preparation was placed in incubation medium containing 20 μM Quin 2-AM. Carbachol (20 μM) was added to the cuvette, and fluorescence measurement was carried out in a Spex Fluorolog, Model 1681 (Spex Industries Inc., Edison, NJ USA) equipped with a water jacket to maintain the temperature at 37°C. A magnetic stirrer was used to keep the cells in suspension. Excitation and emission wavelengths were 340 and 490 nm, respectively. A 50-sec initial fluorescence measurement was carried out before addition of the agonist. Agonist-stimulated rise in $[Ca^{2+}]i$ in calcium-free medium was also measured in random samples of submandibular gland acini (three pairs of glands per dietary group). Ethylene glycol-bis (β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA, 5 mM) was added to the normal medium 30 sec prior to addition of carbachol.

The vitamin C status of the guinea pigs was monitored by measuring the plasma level of total ascorbic acid.[25] Vitamin C content of submandibular gland was also measured in a few random samples of Groups A and B animals.

Statistical analysis

The data were reported as group means ± standard error. The means were compared using, where applicable, the Bonferrono-corrected *t*-tests, and the nonparametric version of one-way ANOVA (the Kruskal–Wallis test). A P value <0.05 was required for us to consider any group differences as statistically significant.

Results

The ascorbate deficient guinea pigs showed marked ($P < 0.001$) reduction in plasma and submandibular gland concentrations of the vitamin compared with findings in control animals with comparable body and gland weights (*Table 2*). A prominent reduction in the stimulated whole saliva flow rate was associated with ascorbate deficiency.

In the ascorbic acid–deficient group, the mean carbachol-stimulated increase in IP_3 generation (120%) was significantly lower ($P < 0.025$) than the mean level (236%) for the adequately fed guinea pigs (*Table 2*). *Figure 1* shows the typical elution profiles for the well-fed (A), ascorbate-deficient (B), and ascorbate-deficient refed adequate diet for 7 days (C). In the three dietary groups, most of the total radioactivity (>85%) in the agonist-generated myo-inositol phosphates (IPS) was recovered in the peak labeled IP_1 than in the other peaks, and this pattern was less evident in the elution profile for the ascorbate-deficient animals (*Figure 1B*). The profiles (*Figure 1*) showed evidence of poor separation between the IP_1 peak and a preceding fraction, and the latter was most likely glycerophosphoinositol since myo-inositol was not retained on the column.[24] IP_1 was

Table 2 Saliva flow rate and levels of inositol trisphosphate (IP_3), cytosolic Ca^{2+} and ascorbate in control and malnourished guinea pigs

Parameter	Control	Ascorbate deficient	*P* value
Plasma ascorbate (μmol/L)	25.6 ± 2.3 (n:17)	3.9 ± 1.1 (n:17)	<0.001
Submandibular gland ascorbate (μmol/g of wet wt)	35.82 ± 5.11 (n:6)	1.53 ± 0.23 (n:4)	<0.001
Saliva flow rate (μL/min)*	80.0 ± 8.1 (n:11)	35.0 ± 6.0 (n:14)	<0.025
IP_3 (percent increase over basal levels)	236 ± 32 (n:6)	120 ± 11 (n:6)	<0.025
Cytosolic Ca^{2+}(nM)	578 ± 36 (n:7)	292 ± 31 (n:11)	<0.01

*Stimulated whole saliva flow rate was determined in guinea pigs not used for studies of IP_3 generation and $[Ca^{2+}]i$ elevation. IP_3 and $[Ca^{2+}]i$ were measured using freshly dispersed submandibular gland acini as described in the text. Data are means ± SEM for the number of animals indicated (n), and each animal with a different cell preparation.

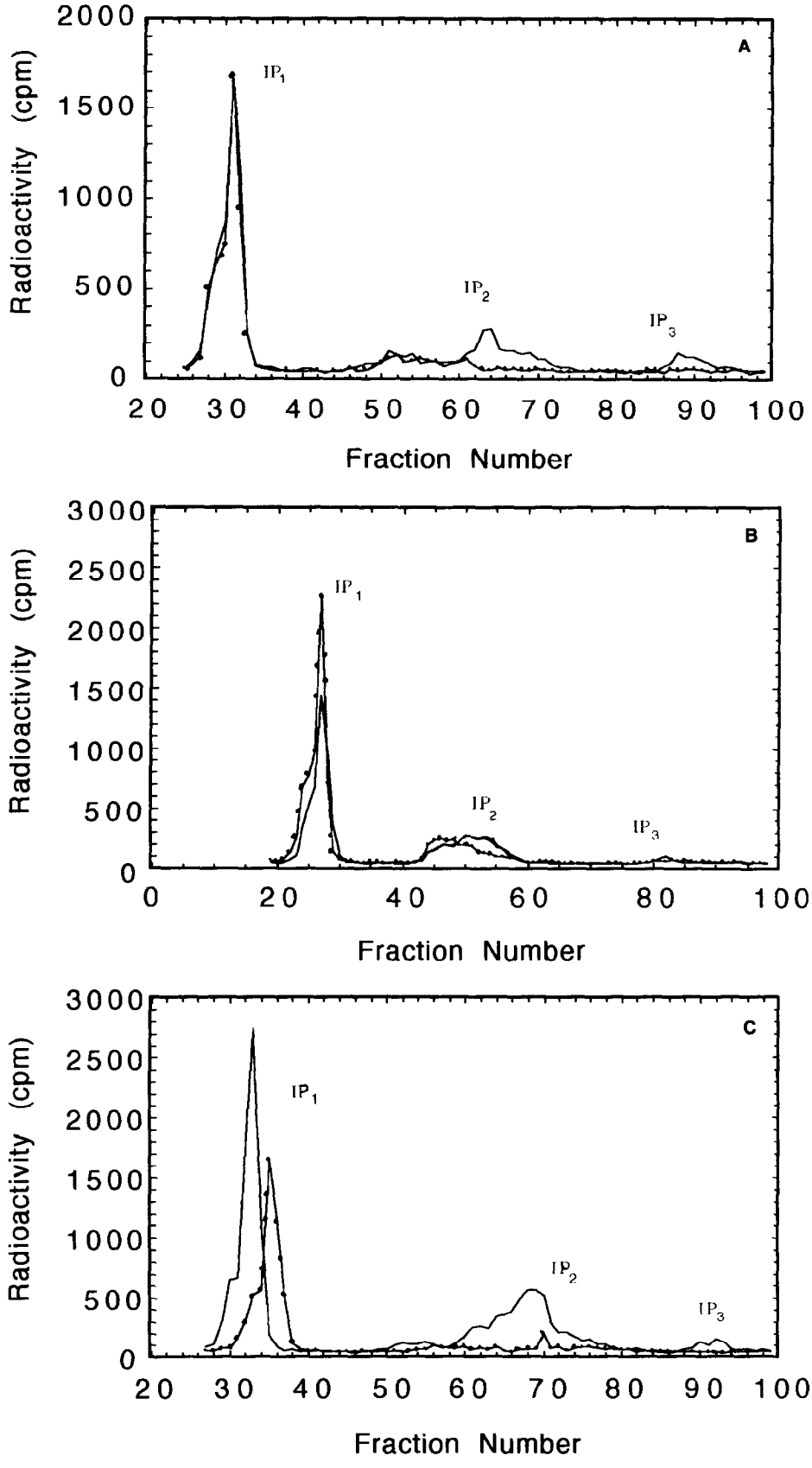

Figure 1 Elution profiles of water-soluble extracts of submandibular salivary glands from adequately fed guinea pigs (A), ascorbate-deficient guinea pigs (B), and deficient guinea pigs refed adequate vitamin C diet for 7 days (C). The washed acinar cells were suspended in 2 mL of HBSS-H buffer containing 40 μCi/mL of myo [^{3}H]-inositol and incubated at 37°C for 80 min with gassing every 20 min. The detailed procedures are as described in the methods section. —●—●—●—●—●— media (blank); —— carbachol stimulated.

therefore identified as glycerophosphoinositol plus myo-inositol 1-phosphate, and IP_2 and IP_3 as myo-inositol 1,4-bisphosphate and inositol 1,4,5-trisphosphate respectively.[24,26] Relative to the IP_1 and IP_2 peaks, stimulated IP_3 peak was consistently very low, particularly in the deficient group (*Figure 1B*). In three ascorbate-deficient guinea pigs refed the vitamin-adequate diet for periods varying from 2 to 7 days (mean of 5 days), the increase in IP_3 generation was 188% (not shown in *Table 2*). The rapid recovery in the refed group is typified by *Figure 1C*.

Associated with impaired carbachol-mediated IP_3 generation in ascorbic acid deficiency was a prominent reduction (<0.01) in stimulated peak $[Ca^{2+}]i$ (292 ± 31 compared with 578 ± 36 mM in control animals) (*Table 2*). The mean resting $[Ca^{2+}]i$ in the deficient group was 70% of the control value (262 ± 13 versus 333 ± 25 nM) (*Figure 2*), and this difference between the two dietary groups was not statistically different. Thus, carbachol-stimulated increases (%) in $[Ca^{2+}]i$ relative to the unstimulated resting $[Ca^{2+}]i$ levels were 73.5 and 11.4 in the control and ascorbate-deficient samples, respectively. These $[Ca^{2+}]i$ levels (*Table 2*) were not corrected for possible mobilization of extracellular Ca^{2+} from the incubation medium. Preliminary studies in our system showed that using EGTA-treated incubation medium, carbachol (20 μM) elicited very transient insignificant increase (6-15%) in $[Ca^{2+}]i$ relative to the resting levels with the increase more pronounced in the deficient group. Additionally, we were more interested in mean differences between two dietary groups processed under identical conditions.

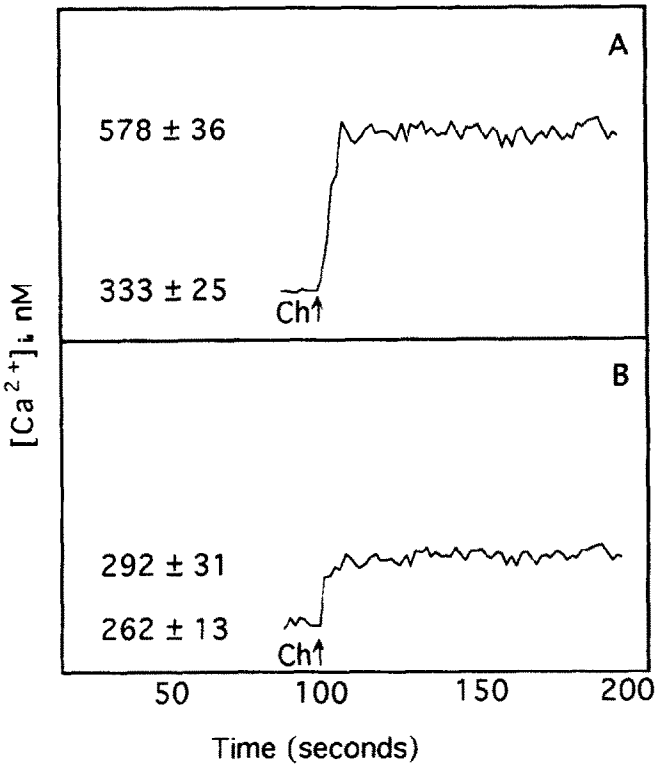

Figure 2 Carbachol (20 μM)-induced $[Ca^{2+}]i$ changes in submandibular gland acini from control (A) and ascorbate-deficient guinea pigs. The submandibular gland acini were prepared and loaded with Quin 2-AM (20 μM). Other details are as described in the methods section. The figure depicts typical tracings, and the values reported for $[Ca^{2+}]i$ represent mean resting and stimulated levels for 7 control and 11 ascorbate-deficient guinea pigs.

Discussion

The model of vitamin C deficiency employed in this study permitted normal food intake by the guinea pigs,[22] thus circumventing the complications of reduced intake of calories, proteins, and other essential nutrients. This approach was necessary in order to isolate the specific effects of ascorbate status. It is known that many dietary factors, including various forms of malnutrition, affect salivary gland secretory function.[27] Protein-energy deficiency (PEM) for example causes significant reduction in the stimulated whole saliva flow rate in humans,[28] an observation confirmed in experimental animal prototypes of PEM which in addition, show a marked decrease in β-adrenoceptor densities in both parotid and submandibular glands with no change in dissociation constants.[18,27] Protein deficiency also impairs incorporation of $NaH_2^{32}PO_4$ into phosphorylated derivatives of inositol in rodent exocrine pancreas,[29] a secretory organ similar in many respects to the major salivary glands. Similarly, dietary fatty acid types influence membrane phospholipids and transmembrane signaling in the rat submandibular salivary gland.[17,30]

The mean concentration of vitamin C in the submandibular glands of adequately fed guinea pigs noted in this study (*Table 2*) was comparable to values reported in the same species by others,[13] thus confirming reports of high sequestration of this nutrient in salivary glands.[12] Next to the adrenal and pituitary glands, the major salivary glands are among the tissues with the highest concentrations of this vitamin in the active, reduced form.[12,13,31] The glandular ascorbate concentration in the deficient guinea pigs was only 4.4% of the control level (*Table 2*). The ascorbate-dependent processes occurring in exocrine tissues are not clearly defined. There is evidence, however, that high intracellular ascorbate affects secretory processes.[11,12,32] In various cell types studied, ascorbate is reported to increase cAMP, partly through reversible inhibition of cyclic adenosine 3′,5′-monophosphate phosphodiesterase (EC 3.1.4.17),[33,34] stimulate active transport of Cl^-,[34] enhance levels of guanosine 3′,5′-cyclic monophosphate (cGMP) several-fold in lymphocytes[14] and platelets,[35] and modulate many cellular events involving participation of calcium ion.[15] Although disputed by some workers,[3] production of cGMP in particular is usually associated with signal-induced phospholipid turnover and elevation of $[Ca^{2+}]i$,[36] and this has been confirmed in electrically stimulated parotid and submandibular salivary glands in rats.[37]

This study showed significantly reduced stimulated IP_3 generation in ascorbate-deficient glands (*Table 2*), an observation indicative of impaired cholinergic activity[1,3] and also consistent with a diminished stimulated saliva flow rate in this group of malnourished guinea pigs.[7] The IP_3 changes reported in this study (*Table 2*) reflected the stimulation (%) over basal IP_3 formed in the absence of agonist. The relatively low IP_3 peaks (*Figure 1*) were consistent with

reports by others.[24,25,38,39] Studies using samples from rat cerebrum, guinea pig parotid gland, and other tissues indicate that under conditions of long agonist stimulation, particularly with carbachol, in the presence of lithium (5 mM), and using aqueous extractions to separate individual phosphates, more than 90% of labeled phosphates are eluted as inositol monophosphate (IP_1) with the peak for IP_3 very low.[24,38,39] In a study of the blowfly salivary gland, Berridge[25] has shown that the agonist-stimulated rapid breakdown of phosphatidyl inositol-4,5-bisphosphate is accompanied by accelerated release of IP_3 which attains a peak 5.5-fold the resting level after 5 sec of stimulation but declines to a much lower plateau level just over twice the resting level.

Although the resting $[Ca^{2+}]i$ level was nonsignificantly lower in ascorbate-deficient than in control submandibular gland (*Figure 2*), peak carbachol-stimulated cytosolic Ca^{2+} was markedly reduced ($P < 0.01$) in the former than in the latter dietary group (*Table 2*). Our data confirmed that the muscarinic-cholinergic transmembrane signaling cascade system was influenced by cellular ascorbate status. The precise mechanisms of action of hypovitaminosis C on this system are not clear. Turnover of membrane phospholipids correlates with functional activity of exocrine glands.[29] Ascorbate status affects lipid metabolism,[22] particularly the integrity of cell membranes,[16] and the latter probably through its role in the regeneration of active vitamin E (α-tocopherol) from the vitamin E radical.[40] Vitamin E is the most important free radical scavenger within membranes and lipoproteins.[16,40] Accumulation of lipid peroxides in membranes can destablize the latter, making them leaky to ions. In addition to lipids, membrane proteins are attacked by peroxyl radicals, an action potentially capable of damaging enzymes, receptors, and signal transduction systems. It is also possible that the endocrine dysfunctions which result from ascorbate deficiency,[23,41] can impair the proper functioning of the G proteins (guanine nucleotide-binding regulatory proteins) as recently reported in patients with gain and loss of endocrine function.[42]

Several in vitro studies, using tissue homogenates, have suggested that ascorbate can exhibit pro-oxidative activity in the presence of free transition metal catalysts such as iron, resulting in a reduction in the number of ligand binding sites including mChR but with no change in binding affinity.[43,44] The pro-oxidant effect of ascorbate could be prevented by the addition of an iron chelating agent such as EDTA.[44,45] It must be underscored that with respect to the in vivo situation in healthy animals, most transition metal ions are not free but are rather bound to proteins.[46]

Striking similarities have been noted between some of the biochemical features of ascorbate deficiency and the effects of long-term treatment of animals with adrenocorticotropin.[47] In our guinea pig model of vitamin C deficiency, there was a profound increase in circulating free cortisol,[7] an observation consistent with the reports of others.[48,49] Increased adrenocorticotropin production has been demonstrated to block the phosphorylation of a membrane protein by protein kinase C, and this in turn inhibits formation of phosphatidylinositol-4,5-bisphosphate (PIP_2),[50] the immediate precursor of IP_3. The latter is consistent with reported observation of a significant reduction in the flow rate of saliva following treatment of rats with dexamethasone[27] and also supports our findings (*Table 2*).

Another relevant aspect currently under investigation in our laboratory is the possibility that some of the observed changes might be due to ascorbate deficiency–induced reduction in the activity of dopamine-β-mono-oxygenase (EC1.14.17.3).[7,9,10] Findings in pituitary cells indicate that dopamine (D) receptor activation reduces $[Ca^{2+}]i$, inhibits phosphoinositide breakdown and/or slows the rate of replenishment of PIP_2 following stimulation of phospholipase C.[51]

The saliva flow rate as well as its contents of antimicrobial factors plays a critically important role in the promotion of good oral health.[52] Xerostomia, the subjective feeling of dry mouth, is associated with salivary gland hypofunction, particularly, a reduced saliva flow rate. Stress, smoking, exposure to ionizing radiation, ingestion of several medications (anorectics, anticholinergics, antipsychotics, antihistamines, antidepressants, hypnotics, etc.), protein-energy malnutrition, uncontrolled diabetes, hypercholesterolemia, and several other pathological states belong to a long list of seemingly unrelated conditions implicated in the genesis of xerostomia.[53,54] Virtually all these conditions feature prominently in promoting cellular depletion of and/or increased tissue requirement for ascorbic acid.[22,55,56]

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