Original articles

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MODULATION OF BUCCAL MUCOSAL CALCIUM CHANNEL ACTIVITY BY SALIVARY MUCINS

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The effect salivary mucus glycoproteins on the activity of calcium channel isolated from buccal mucosal cell membranes was investigated. The channel complex following reconstitution into phospholipid vesicles exhibited an active $^{45}Ca^{2+}$ uptake and responded to calcium channel activator, BAY K8644, and the antagonist, PN200—110. The uptake of $^{45}Ca^{2+}$, while only moderately (15%) affected by the intact mucus glycoprotein was significantly inhibited (60—64%) by the acidic mucus glycoprotein fractions. This effect was associated with the sialic acid and sulfate ester groups of the carbohydrate chains. The channel complex in the presence of epidermal growth factor (EGF) and ATP responded by an increase in protein tyrosine phosphorylation of 55 and 170kDa proteins, and the vesicles containing the phosphorylated channels showed a 46% increase in $^{45}Ca^{2+}$ uptake. The phosphorylation and the calcium uptake were susceptible to inhibition by a specific tyrosine kinase inhibitor, genistein. The binding of EGF to buccal mucosal calcium channel receptor protein was also inhibited (36—41%) by acidic mucus glycoprotein. The reduction in binding was dependent upon the presence of sulfate ester and sialic acid groups, as evidenced by the loss of the glycoprotein inhibitory capacity following removal of these groups. The results for the first time demonstrate that salivary mucins actively participate in the modulation of the buccal mucosal calcium channel activity, a process of importance to the preservation of soft oral integrity.

Key words: buccal mucosa, calcium channel, regulation, salivary mucins.

INTRODUCTION

The importance of salivary mucins in the nonimmune defense of teeth against caries is well documented (1—3), but less is known about the role these glycoproteins play in the preservation of oral mucosal integrity. The available data, nevertheless, indicate that salivary mucins enter into a tenacious interaction with oral epithelial surfaces forming protective coating referred to as oral

mucosal mucus coat (1,4—6). This coat constitutes the pre-epithelial element of oral mucosal defense by acting not only as passive physical obstacle, but also as a dynamic functional barrier capable of modulating the untoward effects of oral environment, including bacterial colonization.

Salivary mucins of oral mucosal mucus coat, remaining in intimate association with the epithelial cell surfaces in the oral cavity, could also conceivably play an important role in affecting the processes occurring within the epithelial perimeter of oral mucosal defense. Primary among these processes is the maintenance of intracellular calcium level. Calcium is an important regulatory element for many cellular functions, including contraction, cell differentiation and secretion, and its influx potentiates mucosal injury caused by a variety of noxious agents (7, 8). The calcium entry in most excitatory and secretory cells occurs through a carefully controlled process involving specific voltage or receptor-dependent channels (7, 9). Recently, we demonstrated the presence in mucosal epithelial tissues of receptor-dependent calcium channels and shown that epidermal growth factor (EGF), through stimulation of channel protein phosphorylation, enhances the calcium uptake (10, 11). In this·report, we provide evidence that salivary mucus glycoproteins are actively involved in the modulation of calcium channel activity in buccal mucosa.

MATERIALS AND METHODS

Materials

Human submandibular/sublingual saliva used for mucus glycoprotein isolation was obtained from five adult secretory with blood-group B (2). Male Sprague-Dawley rats were from Taconic Farms, ($[^{45}Ca]CaCl_2$, $(+)-[methyl-^3H]PN200$-110 from New England Nuclear, and $[^{125}I]EGF$ from Amersham. BAY K8644, eggyolk phosphatidylcholine, V. cholerae neuraminidase, bovine serum albumin, and mouse epidermal growth factor (EGF) were supplied by Sigma, and PN200-110 was a generous gift of Dr. Houlihan, Sandoz Research Institute, E. Hanover, NJ. Wheat germ agglutinin-Sepharose was obtained from Pharmacia, and 5-Bromo-4-chloro-3-indole phosphate and nitro blue tetrazolium were from Oncogene. Goat anti-mouse alkaline phosphatase conjugated IgG and O-phospho-1-tyrosine were from Boehringer Mannheim, and tyrosine kinase inhibitor, genistein and anti-phosphotyrosine monoclonal IgG from Upstate Biotechnology.

Buccal mucosal calcium channel isolation

The animals were anesthesized by i. p. injection with 25% urethane (1 ml/100 g body wt.), and buccal mucosal cells were collected by scraping the mucosa with a blunt spatula. Scrapings were placed in ice-cold buffer (2.5 mM Tris-HCl, pH 7.0, 250 mM sucrose, 2.5 mM EDTA, 1 mM phenyl methyl sulfonyl fluoride (PMSF), 100 TIU/ml aprotinin and 1 µg/ml leupeptin), and homogenized (10). The homogenate was centrifuged at 400 × g for 15 min at 4°C and NaCl/MgSO$_4$ was added to the supernatant to form final concentrations of 0.1 mM and 0.2 mM respectively (10). After centrifugation of the supernatant at 40,000 × g for 1 h at 4°C, the membrane pellet was resuspended

in 0.1 M sodium phosphate buffer, ph 7.2. The preparation was centrifuged for 20 min at 10,00 xg at 4°C, and the pellet solubilized using a buffer containing 40 mM Tris-HCl, pH 7.4, 0.5 M NaCl, 20 mM 3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate (CHAPS), 10% glycerol, 100 TIU/ml aprotinin, 1 µg/ml leupeptin, and 1 mM PMSF. After 1 h at 4°C, the mixture was centrifuged at 105,000 × g for 1 h and the resulting supernatant collected, 1 nM of [³H] PN200-110, a dihydropyridine calcium channel receptor antagonist was added and incubated at 4°C for 30 min. The preparation was then applied to a column of Sepharose-bound wheat germ agglutinin, and the [³H] PN200-110 labeled calcium channel protein was eluted with buffer containing 300 mM N-acetylglucosamine (11).

Salivary mucus glycoproteinpreparation

The lyophilized samples of saliva were dissolved in 6 M urea/10 mM sodium phosphate buffer, pH 7.0, chromatographed on a Bio-Gel P-100 column, and the excluded mucus glycoprotein peak collected. After dialysis and lyophilization, the crude glycoprotein was delipidated with chloroform-methanol (4), and separated on a Bio-Gel A-50 column to low and high molecular weight mucus glycoprotein forms (12). After rechromatography, the glycoproteins were subjected to equilibrium density gradient centrifugation in 42% (w/w) CsCl (2), and the fractions containing mucus glycoprotein were pooled, dialyzed against distilled water and lyophilized. Such prepared low and high molecular weight salivary mucin forms were then used for the isolation of neutral and acidic mucus glycoprotein fractions. For this, the clycoprotein form was dissolved in 0.05 M Tris-HCl buffer, pH 6.8, containing 1 mM EDTA and fractinated on a DEAE-Sephacel column (13). The isolated neutral and acidic mucus glycoprotein fractions were collected, dialyzed against distilled water, and lyophilized.

Effect of salivary mucin on EGF binding

The effect of intact and modified salivary mucus glycoprotein preparations on the binding of EGF was assessed following preincubation of 200 µl aliquots of calcium channel preparation (250 µg protein/assay) with the glycoprotein sample (0—120 µg) at room temperature for 30 min. EGF binding assays were carried out by incubating the channel complex samples (200 µg protein/assay) with [¹²⁵I] EGF (0.18 nM) and 5 mM Tris/HCl (pH 7.0), 125 mM sucrose, 75 mM NaCl, 0.5 mM CaCl₂, 0.5% bovine serum albumin (BSA) in a final volume of 200 µ (10). Membrane-bound [¹²⁵I] EGF was separated from the unbound [¹²⁵I] EGF by centrifugation, the pellet was washed with 1 ml of ice-cold buffer, centrifuged, and counted in a gamma counter.

Effect of salivary mucins on EGF-stimulated calcium channel phosphorylation

The solubilized buccal mucosal calcium channel preparations containing 200—250 µg protein were incubated at room temperature for 30 min in 50 mM HEPES buffer, pH 7.6, containing 10 mM MgSO₄ and 1 mM PMSF, with 0 and 100 µg mucus glycoprotein or 0—40 µg/ml genistein, and 2 µM EGF. The phosphorylation was then initiated by the addition of a solution containing 10 µM ATP, 1 mM CTP, 8 mM MnCl₂, 20 mM MgCl₂, and 2 mM sodium vanadate (10, 11). Incubations were maintained for 10 min at 4°C after which the calcium channel preparation was recovered using wheat germ agglutinin affinity chromatography (10). For SDS-PAGE, the reaction mixture was treated with 170 mM Tris-HCl buffer, pH 6.8, containing 10% SDS and 100 mM dithiothreitol, and heated at 100°C for 8 min. Following electrophoresis, the proteins were

electrophoretically transferred onto nitrocellulose membrane. Mouse-anti-phosphotyrosine mon-oclonal IgG (10 g/ml) was used as detecting antibody, and goat anti-mouse alkaline phosphatase conjugated IgG (0.1 µg/ml) as secondary antibody (14).

Reconstitution of buccal mucosal calcium channels

Liposomes were prepared by the method of Mimms et al. (15). Purified [^{3}H] PN200-110 labeled buccal mucosal calcium channel samples in their intact form and following EGF-stimulated phosphorylation were solubilized with a buffer containing 20 mM CHAPS, 5 mM CaCl$_2$, 50 mM Tris-HCl, pH 7.4, and 10% glycerol, and mixed with 1 ml of octylglucoside containing 1% egg yolk phosphatidylcholine. Detergent was removed by dialysis against buffered saline for 24 h at 4°C, thus yielding liposomes. Further purification of liposomes was accomplished by centrifugation through discontinuous sucrose gradient (16).

Effect of salivary mucin on $^{45}Ca^{2+}$ uptake into vesicles

The effect of salivary mucus glycoproteins on the $^{45}Ca^{2+}$ uptake into vesicles containing the reconstituted, [^{3}H] PN200-110 labeled, buccal mucosal calcium channel was measured following vesicles (200 µl) preincubation for 30 min at rom temperature with different concentrations of the intact and modified mucin preparations (0—120 µg). The external divalent cations were removed on Sephadex G-50 column (17), the vesicles (100 µl) were suspended in 0.34 M sucrose, 10 mM MOPS/tetramethylammonium, pH 7.0, and the calcium uptake was initiated by addition of 50 µl of 0.1 M CaCl$_2$ plus 2 µCi of $^{45}Ca^{2+}$. After 20 min incubation at 37°C, the reaction was terminated with 150 mM MgCl$_2$ in 10 mM N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES)-Tris buffer, pH 7.4 and the samples were applied into GF/C Whatman filter (0.22 µm). Following filtration and washing with 5 mM HEPES-Tris buffer, ph 7.4, containing 0.3 M glucose and 5 mM lanthanum oxide, the filter was subjected to $^{45}Ca^{2+}$ measurement (18). The effect of calcium channel receptor antagonist, PN200-110 and that of calcium channel activator, BAY K8644, on the $^{45}Ca^{2+}$ uptake was measured following vesicles preincubation at room temperature for 20 min with 0—0.8 µM of PN200-110 or 0—10 µM BAY K8644 (10, 11).

Analytical methods

The protein content of salivary mucus glycoprotein preparations and that of buccal mucosal calcium channel samples was measured by the method of Lowry et al. (19), and sulfate was determined turbidimetrically (20). The phenol-H$_2$SO$_4$ method was used for monitoring carbohydrates (2). The quantitative analysis of the content and composition of carbohydrates in various mucus glycoprotein fractions was carried out by gas-liquid chromatography (2, 4, 20). Removal of the sulfate ester groups from the glycoprotein preparations was accomplished by acid catalyzed solvolysis (20), and sialic acid was cleaved with neuraminidase (21). All experiments were carried out in duplicate and the results are expressed as means ± SD. Student's t-test was used to determine significance, and p values of 0.05 or less were considered significant.

RESULTS

The solubilized cell membranes of buccal mucosa used for the isolation of dihydropyridine-sensitive calcium channels were prelabeled with [^{3}H] PN200-100, a dihydropyridine calcium channel receptor antagonist, and purified by affinity chromatography on Sepharose-bound wheat germ

agglutinin. Elution of the column with medium buffer containing 300 mM N-acetylglucosamine yielded the [³H]PN200-110 labeled calcium channel protein complex which, following rechromatography, displayed on SDS-PAGE under reducing conditions four major protein bands migrating in the region of 55, 90, 130, and 170 kDa. The functional performance of the isolated buccal mucosal channel preparation was assessed following incorporation of the channel protein into phospholipid vesicles. The reconstituted calcium channel complex responded in a concentration dependent manner to the addition of calcium channel antagonist, PN200-110, as well as to the specific calcium channel activator, BAY K8644 (*Fig. 1*). The maximal inhibitory effect ($p < 0.05$) was attained at 0.4 μM PN200-110 which gave 71% decrease in calcium uptake, while in the case of BAY K8644, maximal enhancement (58%) in $^{45}Ca^{2+}$ uptake occurred at 6 μM.
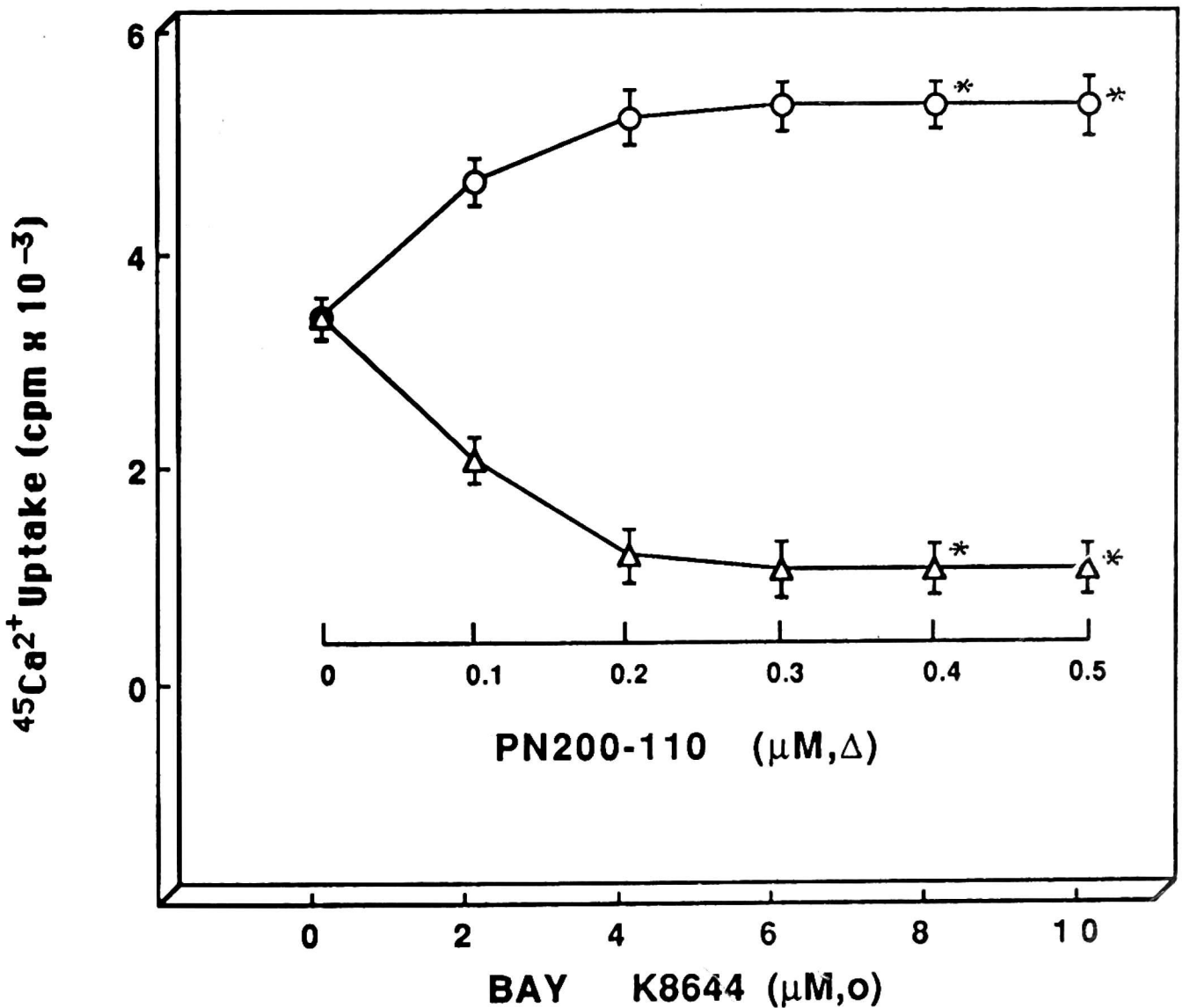

Fig. 1. Effect of PN200-110 and BAY K8644 on the uptake of $^{45}Ca^{2+}$ into vesicles containing the reconstituted buccal mucosal calcium channels. Uptake assays were conducted using vesicles preincubated for 20 min at room temperature with different concentrations of PN200-110 (0—0.8 μM) or BAY K8644 (0—10 μM). Values represent the means ± SD of five experiments performed in duplicate. Asterisk indicates significant change in the uptake of $^{45}Ca^{2+}$.

To evaluate the effect of salivary mucins on $^{45}Ca^{2+}$ uptake into vesicles containing the reconstituted calcium channels, the low and high molecular weight mucus glycoproteins were isolated from human saliva and subjected to fractionation into neutral and acidic fractions. The chemical composition of the low and high molecular weight mucus glycoprotein preparations is given in *Tables 1 and 2*. Preincubation of the reconstituted calcium channels with the intact high and low molecular weight mucins produced in each case about 15% inhibition in $^{45}Ca^{2+}$ uptake, while the corresponding neutral mucin fractions had no discernible effect at all (*Fig. 2*). However, the acidic low molecular weight mucin fraction at its optimal concentraction (80—100 µg/ml) caused a 40% ($p < 0.05$) inhibition in $^{45}Ca^{2+}$ uptake, and the high molecular weight acidic mucin fraction evoced about 60% inhibition ($p < 0.05$) at its optimal concentration of 80—100 µg/ml.

Table 1. Chemical composition of the low molecular weight human salivary mucin and its neutral and acidic fractions

Component (mg/100 mg) mucin	Intact mucin	Mucin fraction	
		Neutral	Acidic
Fucose	12.3 ± 1.0	12.5 ± 3.2	10.9 ± 1.2
Galactose	24.9 ± 2.2	25.6 ± 2.4	23.5 ± 2.5
N-Acetylgalactosamine	11.8 ± 1.3	12.7 ± 1.3	11.3 ± 1.2
N-Acetylglucosamine	17.6 ± 1.7	18.9 ± 1.8	16.1 ± 1.6
Sialic acid	3.9 ± 0.4	0.3 ± 0.2	8.1 ± 0.9
Sulfate	3.5 ± 0.3	0.2 ± 0.1	6.6 ± 0.7
Protein	21.8 ± 2.4	23.2 ± 2.5	20.1 ± 2.4

Each value represents the mean ± SD of three analyses performed in duplicate.

Table 2. Chemical composition of the high molecular weight human salivary mucin and its neutral and acidic fractions

Component (mg/100 mg) mucin	Intact	Mucin fraction	
		Neutral	Acidic
Fucose	13.6 ± 1.1	14.2 ± 1.3	13.0 ± 1.2
Galactose	26.8 ± 2.3	27.1 ± 2.6	24.2 ± 2.1
N-Acetylgalactosamine	13.7 ± 1.0	14.5 ± 1.5	12.6 ± 2.1
N-Acetylglucosamine	23.4 ± 1.8	25.3 ± 2.0	22.1 ± 2.2
Salic acid	3.8 ± 0.4	0.3 ± 0.1	7.9 ± 0.9
Sulfate	3.6 ± 0.5	0.3 ± 0.2	6.8 ± 0.8
Protein	14.1 ± 1.6	15.2 ± 1.6	13.2 ± 1.5

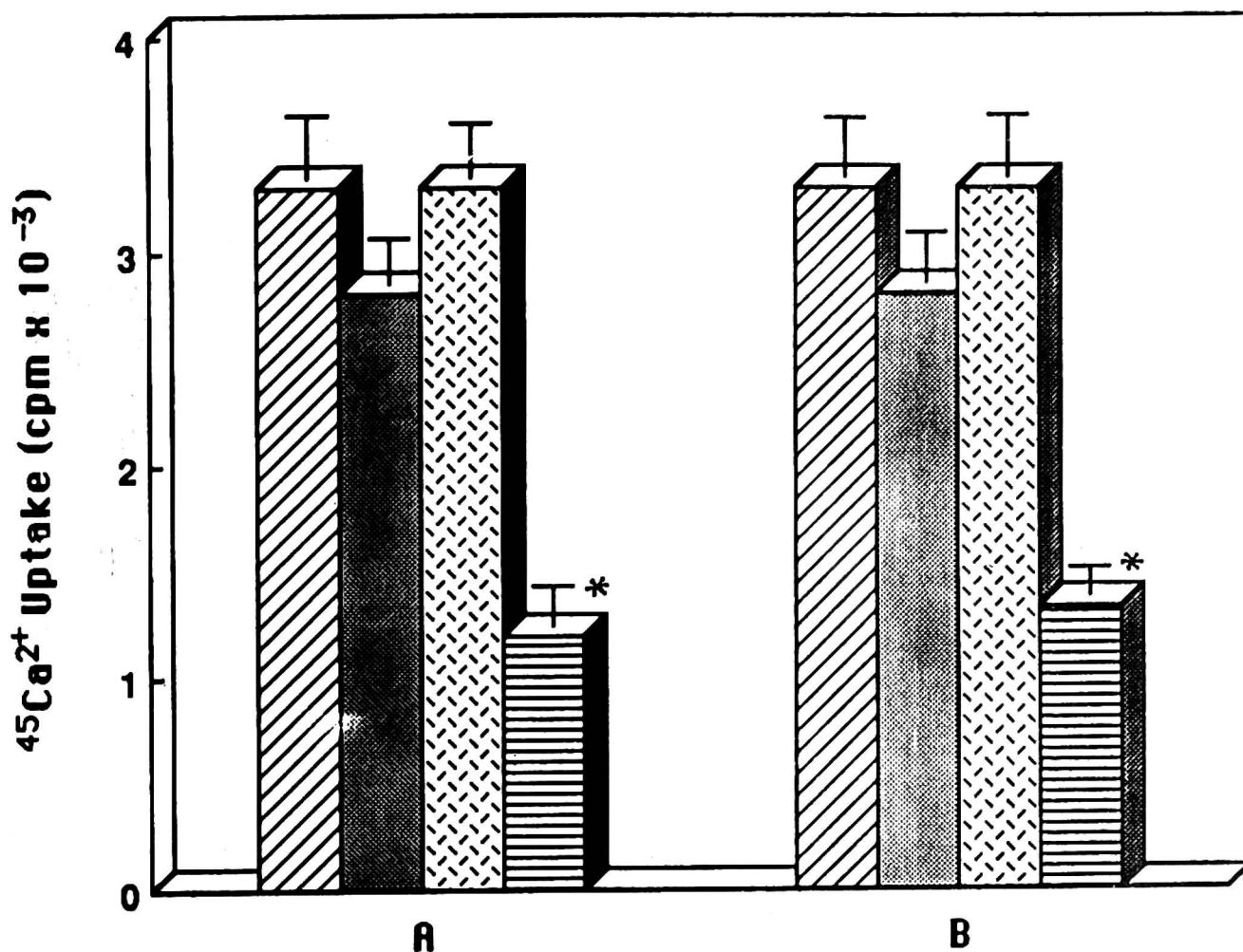Each value represents the mean ± SD of three analyses performed in duplicate.

Fig. 2. Effect of human salivary mucins on $^{45}Ca^{2+}$ uptake into vesicles containing the reconstituted buccal mucosal calcium channels. Uptake assays were conducted using vesicles preincubated for 30 min at room temperature with 0 or 100 μg/ml of intact low (A) and high (B) molecular weight mucus glycoproteins (▨), and their neutral (▨) and acidic (▤) fractions. Control (▨) Values represent the means ± SD of five experiments performed in duplicate. Asterisk indicates significant change in $^{45}Ca^{2+}$ uptake.

The effect of sialic acid and sulfate ester group removal on the $^{45}Ca^{2+}$ uptake vesicles containing the reconstituted buccal mucosal calcium channel is presented in *Fig. 3*. The enzymatic cleavage of sialic acid from the low and high molecular weight acidic mucus glycoprotein fractions caused a 21% decrease in the low molecular weight glycoprotein inhibitory effect on the $^{45}Ca^{2+}$ uptake, and a 17% decrease in the inhibitory effect occurred with the high molecular weight glycoprotein. Desulfation of the glycoproteins produced in the case of the low molecular weight mucin a 41% decrease (p < 0.05) in the inhibitory effect on $^{45}Ca^{2+}$ uptake, while in the case of the high molecular weight acidic mucin, a 38% decrease (p < 0.05) in the inhibitory effect was observed. In both cases, nearly complete loss in the acidic glycoprotein inhibitory effect on the $^{45}Ca^{2+}$ uptake occurred following the desialylation and desulfation.

Fig. 4 presents the data on the effect of salivary mucins on the EGF binding to buccal mucosal calcium channels. The results revealed that the low and high
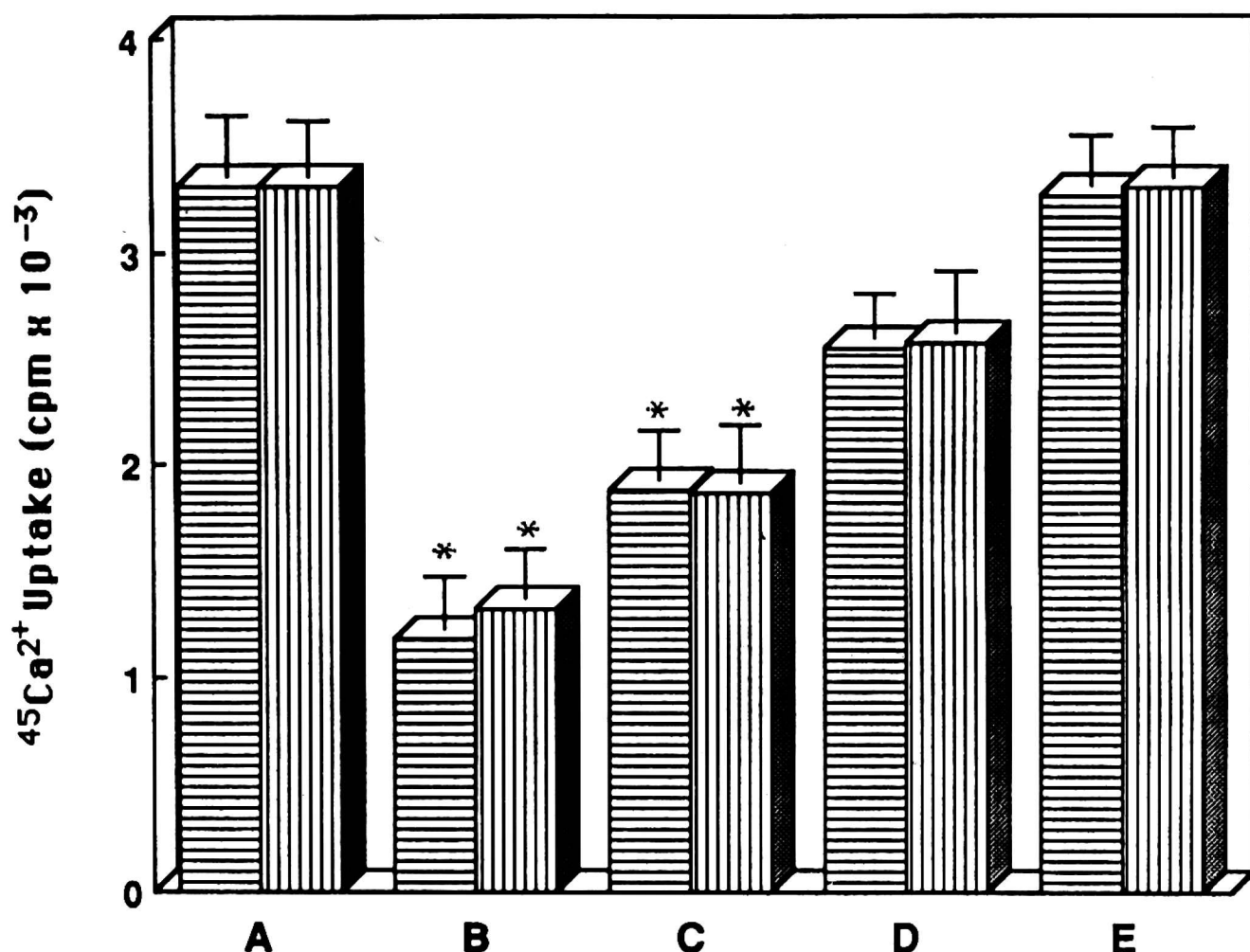
Fig. 3. Effect of desulfation and sialic acid removal on salivary mucus glycoproteins inhibition of $^{45}Ca^{2+}$ uptake into vesicles containing the reconstituted buccal mucosal calcium channels. Uptake assays were conducted using vesicles preincubated for 30 min at room temperature with 100 μg of the low (▤) and high (▥) molecular weight acidic mucus glycoprotein preparations. A, control; B, acidic mucin fraction; C, acidic mucin following removal of sialic acid; D, acidic mucin following desulfation; and E, acidic mucin following desulfation and desialylation. Values represent the means ± SD of five experiments performed in duplicate. Asterisk indicates significant change in the uptake of labeled calcium.

molecular weight neutral salivary mucin fractions over the concentration range (0—120 μg/ml) tested had virtually no effect on the receptor binding of EGF, and the intact mucins produced only 5—7% decrease in the EGF binding. A significant reduction in the receptor binding of EGF was, however, attained with the low and high molecular weight acidic mucin fractions. This inhibitory effect of the acidic low molecular weight mucin reached a value of 41.2% and that of the acidic high molecular weight mucin 36.1%, at the glycoproteins concentrations of 80—100 μg/ml. Following removal of sialic acid, the inhibitory effect of both the low and high acidic mucus glycoproteins on the EGF binding decreased to about 28%, desulfation reduced the inhibitory effect of the glycoproteins to 12—14%, while the desulfated and desialyzed glycoproteins totally lost their inhibitory effect o the buccal mucosal receptor binding of EGF.

The calcium uptake into vesicles containing the reconstituted intact and EGF-induced phosphorylated buccal mucosal calcium channels is shown in *Fig. 5.* Preincubation of the channel preparations with EGF resulted in
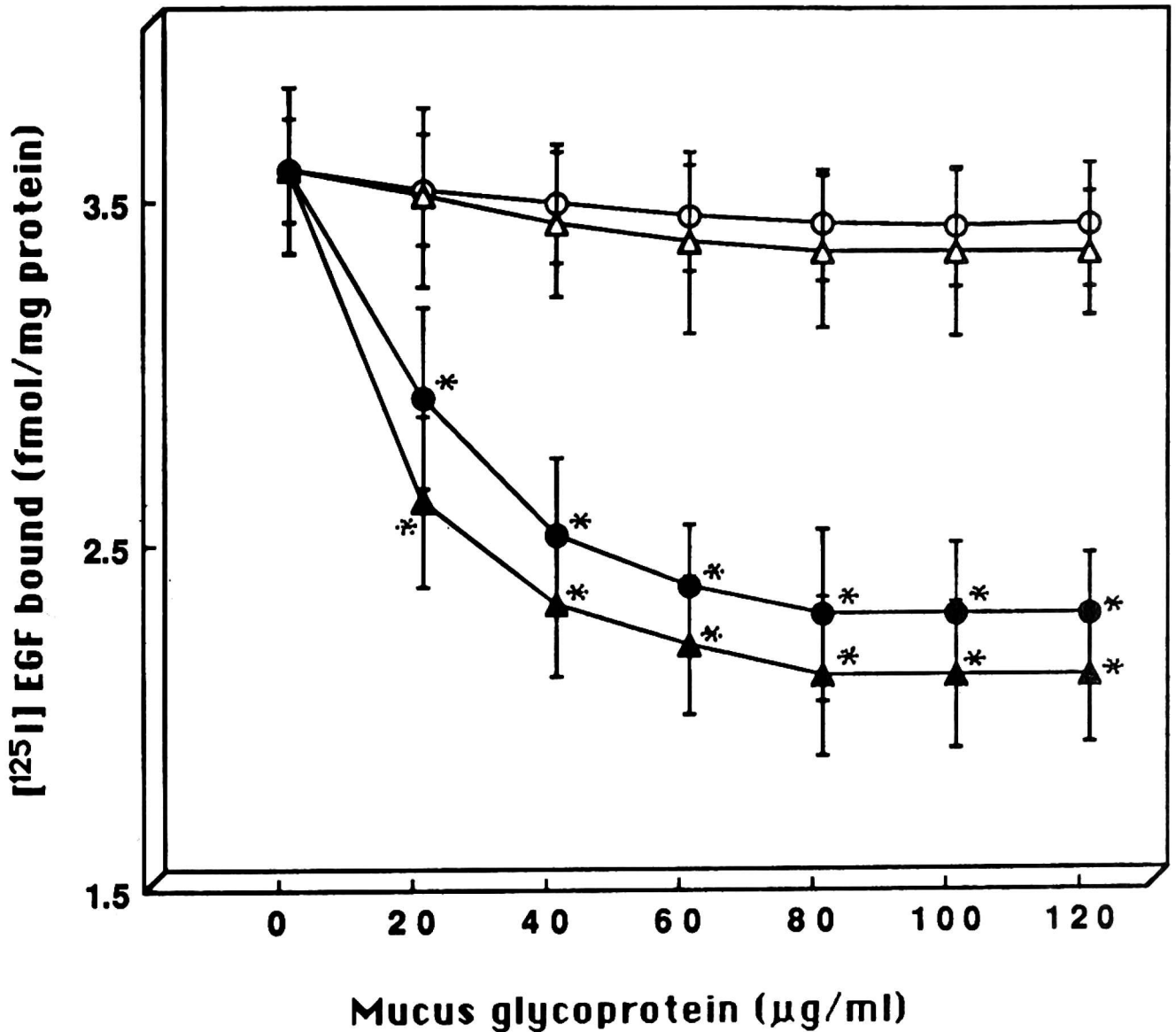

Fig. 4. Effect of the low and high molecular weight salivary mucus glycoproteins on the EGF binding to buccal mucosal calcium channels. Assays were conducted using channel preparations preincubated prior to binding for 30 min at room temperature with 0—120 μg of the glycoprotein. Low molecular weight intact (△) and acidic (▲) mucin. High molecular weight intact (o) and acidic (●) mucin. Values represent the means ± of four experiments performed in duplicate. Asterisk indicates significant change in ^{125}I-EGF binding to mucus glycoprotein.

phosphorylation of the channel proteins, particularly those in the region of 55 and 170 kDa, and the vesicles containing the phosphorylated calcium channels exhibited a 46% greater $^{45}Ca^{2+}$ uptake. The calcium uptake was, however, inhibited by genistein, a known specific tyrosine kinase inhibitor (22), which also caused the inhibition in 55 and 170 kDa channel protein phosphorylation.
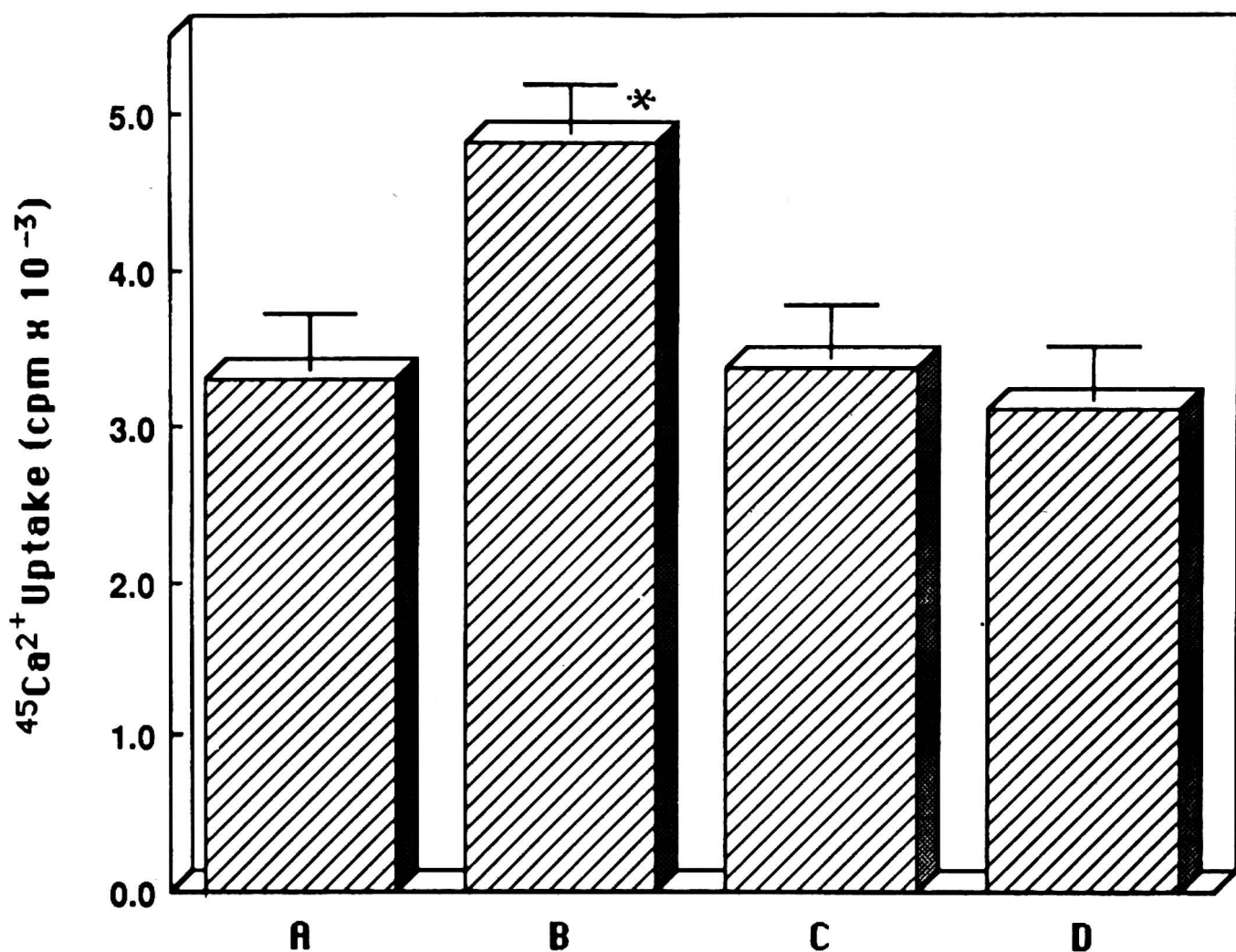
Fig. 5. Effect of EGF-stimulated channel protein phosphorylation on $^{45}Ca^{2+}$ uptake into vesicles containing the reconstituted buccal mucosal calcium channels. Purified channels were reconstituted into phospholipid vesicles either in their intact form (A) or following EGF-induced phosphorylation in the absence (B) and the presence (C, D) of tyrosine kinase inhibitor, genistein 10 μg/ml (C) and 40 μg/ml (D). Values represent the means ± SD of four experiments performed in duplicate. Asterisk indicates significant increase in $^{45}Ca^{2+}$ uptake.

DISCUSSION

Salivary mucins are recognized as a major factor in the nonimmune defense of teeth and oral mucosa against mechanical, chemical and microbial insults (1—6). However, until now, their role has been ascribed primarily to the preservation of oral mucosal integrity at the pre-epithelial level (1). The data obtained in the study presented here demonstrate for the first time that the human salivary mucus glycoproteins have the ability to affect directly the processes occurring within the epithelial cells of buccal mucosa, namely, the maintenance of intracellular calcium level.

Using calcium channels, isolated from buccal mucosal cell membranes and reconstituted into phosphatidylcholine vesicles, we found that salivary mucins
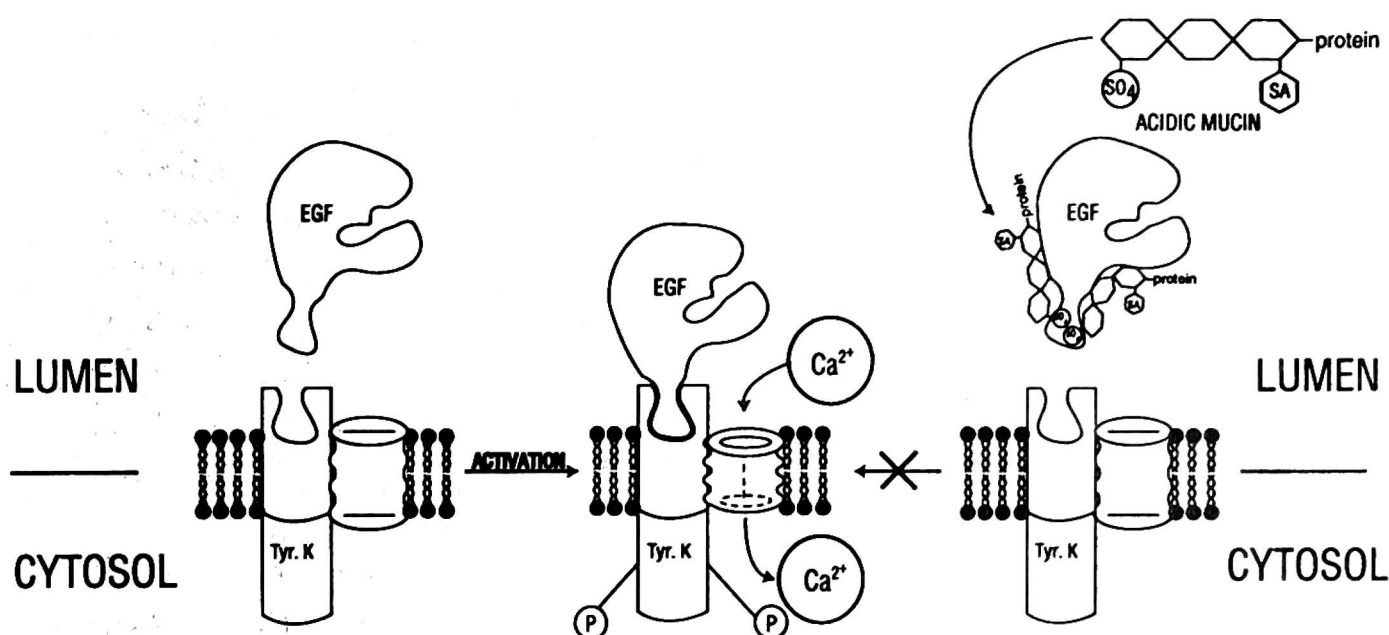
Fig. 6. Schematic representation of the inhibitory mechanism of acidic salivary mucus glycoproteins in the EGF-induced buccal mucosal calcium channel activation. Binding of EGF to its receptor in the calcium channel complex results in the activation of tyrosine kinase (Tyr. K) leading to the calcium channel protein phosphorylation and stimulation of calcium uptake. Acidic salivary mucus glycoproteins by interacting with EGF, apparently interfere with EGF binding to its receptor, and thus exert inhibitory effect on tyrosine kinase activation and channel protein phosphorylation, causing decrease in calcium uptake. SA — sialic acid; SO_4 — sulfate ester group on the carbohydrate chains of mucin.

are capable of modulating the calcium channel activity of soft oral tissue. This inhibitory effect of salivary mucins is associated with the acidic glycoprotein fraction. While the intact mucins at their optimal concentration of 120 µg/ml caused only about 15% inhibition in the uptake, the low and high molecular weight acidic mucin fractions at their optimal concentrations of 80 µg/ml evoked 54% and 60% inhibition, respectively in the uptake of $^{45}Ca^{2+}$ into the vesicles containing the reconstituted buccal mucosal calcium channels.

The demonstrated inhibitory effect of the low and high molecular weight acidic salivary mucus glycoproteins on buccal mucosal calcium channel activity is apparently due to the presence of sialic acid residues and sulfate ester groups on the carbohydrate chains, as the removal of either sialic acid or sulfate caused partial loss in the glycoproteins inhibitory effect, and a complete loss in the inhibitory effect on $^{45}Ca^{2+}$ uptake occurred following desialylation and desulfation of the glycoproteins. The regulatory effect on calcium channel activity has also been reported in other systems for such acidic glycoconjugates as heparin and GM_1-ganglioside (10, 23). Hence, the identified ability of acidic salivary mucins to modulate the buccal mucosal calcium channel activity could be viewed as yet another feature of the multifunctional role the salivary mucus glycoproteins play in the preservation of oral mucosal integrity.

Since, as reported recently with patch clamp recordings (24), the activation of the EGF receptor triggers the receptor-operated calcium channel response,

and since it has been shown that phosphorylation of calcium channel protein triggered by EGF receptor activation enhances calcium uptake (10, 11), the effect of salivary mucus glycoproteins on the phosphorylation event and the expression of channel activity was investigated. While the intact low and high molecular weight mucus glycoproteins had only negligible effect (5—7%) on the receptor binding of EGF, the corresponding acidic mucin fractions caused a significant reduction (41.2% and 36.1%) in the EGF binding. This effect of the low and high molecular weight acidic glycoproteins apparently depends upon the presence in their carbohydrate chains of both sialic acid and sulfate ester groups, as the desulfated and desialyzed glycoproteins totally lost the inhibitory effect on the buccal mucosal receptor EGF binding.

The calcium channels on the EGF binding in the presence of ATP responded by an increase in protein tyrosine phosphorylation, reflected mainly in 55 and 170 kDa proteins, and the vesicles containing such phosphorylated channels showed about 46% higher $^{45}Ca^{2+}$ uptake. The uptake of calcium was, however, inhibited by a known protein tyrosine kinase inhibitor, genistein (22). This tyrosine-specific kinase inhibitor, furthermore, caused inhibition in buccal mucosal 55 and 170 kDa channel protein phosphorylation.

The finding that the low and high molecular weight acidic salivary mucins are capable of regulating the buccal mucosal calcium channel activity through the inhibition of EGF-stimulated calcium channel protein tyrosine phosphorylation attests further to the importance of salivary mucins in the regulation of functional expression of EGF in the oral cavity, a process of paramount importance to the maintenance of oral tissue integrity under normal physiological conditions, and its repair following injury. The schematic representation of the inhibitory mechanism of acidic salivary mucus glycoproteins in the EGF-induced buccal mucosal calcium channel activation is depicted in *Fig. 6*. As uncontrolled calcium influx is known to aggravate mucosal injury, our findings provide for the first time a direct evidence for the active participation of salivary mucins in the modulation of the processes occurring in the epithelial perimeter of oral mucosa.

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