Induction of Proline-rich Proteins in Hamster Salivary Glands by Isoproterenol Treatment and an Unusual Growth Inhibition by Tannins*

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(Treatment of hamsters with the β-agonist isoproterenol caused a dramatic increase in a series of unusual proteins in the parotid and submandibular glands. These proteins are acid soluble and they contain high amounts (mol %) of glutamate plus glutamine (30-35), proline (23-30), and glycine (12-25). Three proteins (HP15, HP13a, and HP43b) were isolated from tri-chloroacetic acid extracts of parotid glands of isoproterenol-treated hamsters. The basic protein (HP15) was not retained by DEAE-cellulose and did not contain phosphate or carbohydrate. Two acidic proteins (HP13a and HP43b) had the same apparent molecular weight on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, but these were separated by DEAE-cellulose chromatography. HP13a and HP43b contained 4.3 and 5.7 phosphate residues/mole of protein, respectively. Levels of mRNAs encoding this series of proteins showed striking increases following isoproterenol treatment as determined by cell-free translations and Northern analysis. Feeding tannins to rats and mice mimics the effects of isoproterenol treatment on the parotid gland (Mehansho, H., Hagerman, A., Clements, S., Butler, L., Rogler, J., and Carlson, D. M. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 3945-3952; Mehansho, H., Clements, S., Sheares, B. T., Smith, S., and Carlson, D. M. (1985) J. Biol. Chem. 260, 14118-14233). However, hamsters on a high tannin diet (2%) did not respond like rats and mice and instead displayed an unusual growth inhibition. Weanling hamsters maintained on a 2% tannin diet initially lost weight for 3 days and then failed to gain weight for up to 6 months when kept on this diet. Essentially a normal growth rate was observed when the tannin-fed hamsters were switched to a normal diet.

Salivary glands of various animals can synthesize, or can be induced to synthesize, a group of proteins which are unusually high in proline, the so-called proline-rich proteins (PRPs); (1). These proteins collectively constitute about 70% of the proteins in human salivary secretions (2). The PRPs are encoded by multigene families (1) and can undergo various post-translational modifications including proteolysis, phosphorylation, and glycosylation. These unusual proteins are presumably constitutive in human saliva (2), but families of similar proteins are dramatically increased or induced in parotid and submandibular glands of rats (3, 4), mice (5, 6), and hamsters (this report) by isoproterenol treatment. The nucleotide sequences of several PRP mRNAs from rat (7, 8), mouse (9), and human (10) and the structures and organizations of complete genes of PRP multigene families from the mouse (10), hamster (11), and human (12) have been reported. Proteins derived from the nucleotide sequences are all characterized by four general regions: a putative signal peptide, a transition region, the repetitive region, and a carboxyl-terminal region (1).

Previously we found that feeding tannins mimicked the effects of isoproterenol on parotid glands, causing glandular hypertrophy and induction of PRPs in rats (5) and mice (13). The apparent tissue-specific synthesis and the appearance of PRPs in saliva suggest a biological function in the oral cavity and gastrointestinal tract. Evidence has been presented that these proteins have high affinities for tannins and that they can reverse the detrimental effects of tannins in the diets of rats (13) and mice (5). However, hamsters do not respond to dietary tannins by inducing the synthesis of PRPs and as a result, tannins show an unusual ability to inhibit growth. Tannins are also unusually toxic to hamsters. We have isolated and partially characterized three proteins from hamster parotid glands which are high in glutamate (or glutamine), proline, and glycine and which are dramatically induced by isoproterenol treatment. In an attempt to determine the reasons for the different responses to tannins, assays for β-adrenergic receptors and adenylate cyclase were performed on membrane preparations from parotid glands of rats and hamsters. Cell-free translations and Northern analysis were carried out on control and isoproterenol-treated animals to determine the extent of induction of PRP mRNAs.

EXPERIMENTAL PROCEDURES

Materials—All materials were of highest purity available and were purchased from commercial sources unless otherwise indicated. The following were purchased from respective suppliers: 1-[3,4-3H] proline (100 Ci/mmol), gelatin, ICN or Nutritional Biochemicals; 3-[3H] iodoacetylmethyl (200 Ci/mmol), Amersham Corp.; dl-isoproterenol; HCl, ATP, CAMP, lysozyme, phosphoenolpyruvate, pyruvate kinase, binding protein, chymotrypsin, Sigma; ENHANCY, Du Pont.

*The abbreviations used are: PRP, proline-rich protein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; H29, hamster proline-rich protein gene; CNBr, cyanogen bromide.
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New England Nuclear; molecular weight standards, Bio-Rad; trypsin-
tosylphenylalanine chloromethyl ketone, clestrain, Worthington; 
Staphylococcus aureus protease, Miles Laboratories Inc.; Quebracho 
extract, Fask Chemical Co.; reticulocyte lysate, gift from Dr. David 
Kuhn, Department of Biochemistry, Purdue University; high tannin 
sorghum (Savanna), and low tannin sorghum (RS-610) were grown 
at the Purdue University Agronomy Farm.

Feeding Trials—Male Golden Syrian hamsters (50-60 g) were 
maintained on Purina Lab Chow for 5-6 days before starting the 
feeding experiments. Sorghum diets were prepared as described else-
where (13). Feed and water were provided ad libitum. Tannin contents 
were measured by a competitive binding assay (14). The condensed 
tannin contents of Savanna sorghum, grain and Quebracho extract 
were 2 and 50%, respectively, Gelatin, Quebracho extract, or an amino 
acid mixture equivalent to gelatin in amino acid composition was added 
to sorghum diets as indicated at 4% of the diet weight.

Isoprotorenol Treatment—Male Golden Syrian hamsters (90-100 
gf) fed Purina Lab Chow were utilized as gland donors. Each hamster 
was injected intraperitoneally with the indicated amount of dl-isoprot-
enol-HCl daily as described by Muenter et al. (13). Unless otherwise 
indicated, hamsters were treated daily with 1.0 mg of isoprotorenol 
for 10 days.

Isolation of Proline-rich Proteins—Animals were anesthetized with 
sodium pentobarbital and killed by exsanguination. The parotid and 
submandibular glands were removed and stripped of connective tissue 
and fat. Trichloroacetic acid extractions were performed as described 
by Melander and Carlson (14). The acid-soluble fraction, which contains 
most of the proline-rich proteins, was further fractionated by DEAE-cellulose chromatography. About 2 mg of sample was 
reconstituted in a column (1.5 x 70 cm) which had previously been equili-
ibrated with 5 mm phosphate buffer, pH 8.0. After washing with 150 
ml of the equilibrating buffer, elution was carried out with a linear gradient 
ranging from 0 to 1 M NaCl. Membrane Preparation—Crude membranes were prepared as 
described by Ludford and Talano (15) with a slight modification. Fresh 
parotid glands from normal rats and hamsters were homogenized in 
20 volumes (mg/ml) of buffer A (125 mM sodium phosphate, pH 7.4, 
0.3 M NaCl, and phenylmethylsulfonyl fluoride (15 mg/ml) for 30 s, 
oven centrifugation was centrifuged at 31,000 g for 1 h. One-half of 
the pellets were suspended in the same volume of Buffer A (10 mg/ml) 
in tissue with a Daum homogenizer. This crude membrane preparation 
was used for the 123I-hydroxybenzylidol binding experiments. The 
remainder of the pellets was suspended in 4 volumes of cAMP 
assay buffer (50 mM Tris-HCl, pH 7.5, 4 mM EDTA), and this 
membrane preparation was used for assaying adenyl cyclase activ-
ity.

β-Adrenergic Receptor Binding Assay—The ability of the mem-
brane preparations to bind a β-agonist was determined by using 123-
I-hydroxybenzylidol (15). A final volume of 1 ml containing 0.5 ml 
of membrane suspension, 0.2 mg of lysosome, 10 mM GlnAc, and 
200 pmol of 123I-hydroxybenzylidol. The assay mixture was in-
cubated at 37 C for 1 h. Assays were carried out in the presence 
and absence of 10 mM dl-propranolol. Membranes were collected on 
glass-fiber filters, and bound 123I-hydroxybenzylidol was measured in 
a γ counter.

Adenyl Cyclase Assay—Adenyl cyclase activities of parotid 
membrane preparations were measured according to the procedures 
of Ludford and Talano (16) and ToweY et al. (11). Assay mixtures 
(final volume, 100 μl) consisted of 40 mM Tris-HCl (pH 7.4), 10 mM 
Mg2+, 10 mM theophylline, 0.1 mM EDTA, 10 mM phosphoenolpy-
pyruvate, 25 μg of pyruvate kinase, 1 mM ATP, and 50 μl of membrane 
suspension. These were incubated for 10 min at 37 C. Amounts of 
cAMP generated were estimated by the competitive protein binding 
technique (17).

Protein Determination—Proline-rich proteins usually lack aromatic 
aminic acids and are estimated by absorbance at 230 nm. The 
calculated extinction coefficient for one of these proteins is ε520 = 
25 (4). Protein in membrane preparations was measured by the Lowry 
method (18) using bovine serum albumin as a standard.

Amino Acid Analysis—Protein samples (20-50 μg) were hydro-
lized and analyzed as described earlier (4).

Protein Sequence Analysis—The amino-terminus of the region 
of three proteins from hamster parotid glands (HP45, HP43a, and HP43b) 
were determined according to Hermodson et al. (19).

Proteolytic Digestion of HP45 and HP43a—Incubation conditions 
for pepsine treatments were: clostrain, 50 mM ammonium bicar-
bonate, 0.27 M mercaptoethanol, 37 °C, 4 h; S. aureus protease, 50 
mM ammonium acetate, 37 °C, 18 h; trypsin, 0.01 M HCl, 37 °C, 6 h; 
chymotrypsin, 0.5% NaHCO3, 37 °C, 6 h. Enzyme/substrate ratios 
(w/w) for clostrain, S. aureus protease, and chymotrypsin were 1:50, 1:50, and 1:50, respectively. Cyanogen bromide diges-
tion was performed as described (20).

Isolation and Analysis of RNA—Total RNA was prepared by 
using the guanidine thiocyanate-cesium chloride procedure described 
in Chingwu et al. (21). RNA was translated in a rabbit reticulocyte 
lysate system, and the translation products were analyzed as described 
previously (6). Northern blot analysis was performed as described by 
Thomas (22) using electrophoresis in a 5% agarose gel containing 2.2 
mM formaldehyde (23). 5′ Labeled exon III of hamster PRP gene 
H29 (11) was prepared by nick translation and was used as a probe 
for hybridizations. The filters were treated as described previously 
(6).

RESULTS

Effects of Isoprotorenol Treatment—Isoprotorenol treat-
ment has a dramatic hypertrrophic effect on the parotid and 
submandibular glands of rats (3, 4) and mice (5) with the 
values of the sizes of these glands increasing by about 10- and 5-fold, 
respectively, after 10 days of treatment. Increases in sizes of the 
parotid and submandibular glands of hamsters were es-
entially negligible (±0.5-fold), but isoprotorenol treatment of 
Hamsters did induce the synthesis or accumulation of acid-
soluble proteins about 6-8-fold (Table I). As found with rats 
(3) and mice (5), these proteins were high in proline but were 
also unusually high in glutamate (or glutamine) (Table II).

Electrophoretic patterns of acid-soluble proteins and gly-
coproteins induced in salivary glands of hamsters treated with

| TABLE I |
| Effects of isoprotorenol treatment on gland weights and on amounts of acid-soluble proteins |
| Gland weight | Acid-soluble proteins |
| Parotid | Submandibular | Parotid | Submandibular |
| n g | g/kg tissue | % | |
| Isoprotorenol | 0 | 0.32 | 0.70 | 5.5 | 10.7 |
| 0.5 mg | 0.42 | 0.80 | 22.8 | 48.1 |
| 1.0 mg | 0.38 | 0.85 | 29.7 | 83.6 |
| 1.5 mg | 0.40 | 0.80 | 27.6 | 63.5 |
| 2.5 mg | 0.50 | 1.00 | 37.9 | 67.4 |
| 3.5 mg | 0.50 | 0.85 | 31.6 | 83.5 |

| TABLE II |
| Amino acid compositions |
| HP45 | HP43a | HP43b | H29* |
| mol/100 mol | mol/100 mol | mol/100 mol | |
| Asx | 7.9 | 10.3 | 10.8 | 10.7 |
| Thr | 0.1 | 1.4 | 1.6 | 1.2 |
| Ser | 0.6 | 3.7 | 4.2 | 4.7 |
| Glx | 31.7 | 34.1 | 30.7 | 34.9 |
| Pro | 30.8 | 22.3 | 23.5 | 20.1 |
| Gly | 24.8 | 12.2 | 14.1 | 14.2 |
| Ala | ND | 1.9 | 2.3 | 1.7 |
| Ile | ND | 0.5 | 0.8 | 1.2 |
| Leu | 0.5 | 1.3 | 1.4 | 1.8 |
| Tyr | ND | ND | 0.6 | 0.6 |
| His | 0.5 | 3.0 | 3.1 | 1.2 |
| Lys | 1.3 | 5.1 | 3.4 | 3.6 |
| Arg | 0.9 | 3.9 | 3.3 | 3.6 |
| Met | ND | ND | 0.2 | 0.2 |
| Phe | 0.9 | ND | ND | 0.6 |

*mol of PO4/mol of protein 0.43 4.3 5.7

*a Amino acid composition derived from the nucleic acid sequence of hamster PRP gene H29 (11) as given in Fig. 9 and assuming Ala is the N-terminal amino acid of the mature protein.

*b Asx = Arg + Asn.

c Glx = Glu + Gln.

*ND, not detected.
varying amounts of isoproterenol are shown in Fig. 1. The profiles and levels of most proteins were essentially unchanged when the amount of isoproterenol was increased from 0.5 to 3.5 mg/animal. Several proteins are glycosylated, especially in isoproterenol-treated submandibular glands (Fig. 1B). A glycoprotein of $M_{r}$ of 89,000 (GP89) showed an unusual regulatory pattern; it was induced dramatically in parotid glands of hamsters treated with 2.5 mg of isoproterenol/day.

**Protein Isolations**—The acid-soluble proteins extracted with 10% trichloroacetic acid from parotid glands of isoproterenol-treated hamsters were partially resolved by DEAE-cellulose chromatography (Fig. 2). Fractions from peaks I, III, IV, and V lacked absorbance at 280 nm, which is consistent with results obtained with other PRPs (3). To check for homogeneity, each fraction from peaks I through V was assayed by SDS-PAGE (data not shown). As illustrated in Fig. 2 (inset), peak I which was not retained by the DEAE-cellulose column appears as one protein in SDS-PAGE, and this protein has a $M_{r}$ of 45,000 (HP45). Peak III includes at least 3 proteins. Peaks IV and V contain glycoproteins HP43a and HP43b, each with a $M_{r}$ of 43,000. A minor contaminant of HP43b was removed by repeating the DEAE-cellulose chromatography with a lower salt gradient.

**Composition Analysis**—The amino acid compositions of HP45, HP43a, and HP43b are presented in Table II. These proteins are all high in glutamic acid (or glutamine), proline, glycine, and aspartic acid (or asparagine). They either lack or contain very low amounts of aromatic and sulfur-containing amino acids. HP43a and HP43b are different, but they do have similar amino acid compositions. HP43a and HP43b contain 4.3 and 5.5 mol of phosphate/mol of protein, respectively (Table II). The amino acid composition of the protein encoded by hamster PRP gene H29 (11) is similar to, but not identical with, either HP43a or HP43b.

**Partial Amino Acid Sequences of HP45, HP43a, and HP43b**—Sequences of the amino-terminal regions of HP45, HP43a, and HP43b are compared together with sequence data from proline-rich proteins of rat, mouse, and human (Fig. 3). There is about 73% homology in the first 11 amino acids of HP43a and HP43b. When the sequence of HP43a is compared with the amino acid sequence derived from PRP gene H29 (residues 15-25) (11), there is a single substitution at position 8; aspartic acid (HP43a) (codon GAU) for isoleucine (H29) (codon ATA). When sequences of HP43a and HP43b were aligned with peptides encoded by PRP cDNAs of mouse (pMP125) and rat (pRP33), there were two regions of homology for all four peptides. The basic PRP, HP45, had no homology with either HP43a or HP43b (or with other basic PRPs from rat (8), mouse (8), and human (24)). Both HP43a and HP43b have relatively high amounts of serine and threonine which are potential sites for phosphorylation and glycosylation. For example, residue 9 in HP43a and HP43b is missing from sequence analysis as would be expected if it was either glycosylated or phosphorylated.

**Treatment with Proteases and CNBr**—HP45 and HP43a were treated with various enzymes and with CNBr. A characteristic difference was observed between HP45 and HP43a (Fig. 4). HP45, the basic PRP, was resistant to $S$. aureus protease V8 whereas HP43a was an excellent substrate. Both behaved similarly toward clostripain and trypsin. Chymotrypsin hydrolyzed HP45 extensively, but HP43 appeared to have a single cleavage site. As expected, both HP45 and HP43a were resistant to CNBr.

**Binding Assays of Hamster Proline-rich Proteins to Tannins**—A high affinity for tannins is one of the characteristic properties of PRPs (13). The relative affinities of tannins for four PRPs from hamster parotid glands were measured by a binding assay using $[^{14}C]$ labeled bovine serum albumin (14). These proteins have about an 8-fold greater affinity for tannin than does bovine serum albumin (Fig. 5). Results of these binding assays are comparable to those obtained for rat (13) and mouse '5' PRPs.

**Cell-free Translations**—Total RNAs prepared from parotid glands of isoproterenol-treated and control hamsters were translated in vitro by the reticulocyte lysate system (Fig. 6) in the presence of $[^{14}C]$ labeled proline. Isoproterenol treatment increased demonstrably the in vitro synthesis of several proteins. Many proteins induced by isoproterenol treatment were apparently present normally in low amounts. Two polypeptides (M, 58,000 and a low molecular weight protein) disappeared with isoproterenol treatment. This observation is consistent with the reduction or disappearance of $\alpha$-amylase and parotid-specific protein in cell-free translations of RNAs from isoproterenol-treated rats (7) and mice (6). Cell-free translations with $[^{35}S]$methionine (not shown) clearly demonstrated the dramatic decrease in $\alpha$-amylase.

**Northern Hybridization**—Relative changes of PRP mRNAs after isoproterenol treatment were determined by Northern analysis using exon III of hamster PRP gene H29 (11) as the probe (Fig. 7). Isoproterenol treatment caused about a 10-fold induction in PRP mRNAs. This increase is considerably lower...
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Fig. 2. DEAE-cellulose chromatography of the acid-soluble components from hamster parotid glands. Elution was performed as described under "Experimental Procedures." Analysis of peaks, I, III, IV, and V by SDS-polyacrylamide gel electrophoresis is shown in the inset.

Fig. 3. Comparison of the N-terminal regions. H29, hamster PRP gene (derived sequence) (11); HP45, hamster basic PRP; HP43a and HP43b, hamster acidic PRPs (in this report); pRP33, rat cDNA encoding an acidic PRP (7); pMP125, mouse cDNA encoding acidic PRP (6); protein A, human acidic PRP (2); IB-1, human basic PRP (24); XXX are likely glycosylated or phosphorylated serines or threonines.

Fig. 4. Protease and CNBr treatment of HP45 and HP43a. 1, no enzyme; 2, S. aureus V8; 3, clostripain; 4, trypsin; 5, chymotrypsin; 6, CNBr. Protease digestions and CNBr cleavage were performed as described under "Experimental Procedures." Molecular weight markers are indicated by dashes.
Fig. 5. Competitive binding curves between $^{14}$C-labeled bovine serum albumin (BSA) and hamster PRPs for tannin. Increasing amounts of competitor were mixed with 100 µg of $^{14}$C-labeled bovine serum albumin in 640 µl of 0.2 M acetate buffer (pH 4.8). To this was added 160 µl of methanol containing 20 µg of tannin. After centrifugation, supernatant fluids were removed and discarded. The pellets were washed, dissolved in 100 µl of 1% SDS, and assayed for radioactivity by scintillation counting. Inhibition of binding was calculated as described (14). Competitor proteins added were hamster PRPs: HP80 (■), HP45 (▲), HP43a (▲), HP43b (○), and bovine serum albumin (●).

B N IPR

- 58 K
- 38 K
- 32 K
- 27 K

Fig. 6. Cell-free translations of mRNAs from hamster parotid glands. Translations were carried out with the reticulocyte lysate system and labeled with $^{35}$Hproline. About 5 µl of each translation mixture was analyzed by fluorography. B, no added RNA; N, normal; IPR, isoproterenol treated.

provided by the gelatin and suggests the formation of a tannin-gelatin complex. In contrast, rats maintained on high tannin diets (2'/tannin) survived and gained weight after an initial weight loss (13); the ability to gain weight was clearly the result of induction of PRP synthesis by tannins (13). Undoubtedly the severe adverse effects to hamsters of diets high in tannins are due to the lack of induction of the tissue-specific PRP multigene family. Increasing the tannin content of the high tannin diet from 2 to 4'/tannin by adding Quebracho

Fig. 7. Northern hybridization analysis of RNA from parotid glands of normal and isoproterenol-treated hamsters. RNAs (10 µg) from parotid glands of normal (NOR) and isoproterenol treated (IPR) were electrophoresed, blotted, and hybridized to $^{32}$P-labeled probes. M.W. Std. bp., molecular weight standards (base pairs), prepared by a HaeIII digest of X-174 DNA.

**Table III**

| Diet*       | Gland weight | Acid-soluble proteins |
|-------------|--------------|-----------------------|
|             | Parotid      | Submandibular         | Parotid      | Submandibular |
|             | kg/mg tissue | mg/mg tissue          | kg/mg tissue | mg/mg tissue |
| RS-610      | 0.24         | 0.46                  | 4.1          | 11.0          |
| Savanna     | 0.23         | 0.45                  | 4.6          | 12.2          |

*Hamsters were maintained either on RS-610 (low tannin sorghum) or on Savanna (high tannin sorghum) diets for 3 days.

Fig. 8. Effects of high tannin (Savanna) and low tannin (RS-610) sorghum diets on rate of growth of hamsters. Inset, rate of growth of rats fed Savanna (○—○) and RS-610 (●—●)
**TABLE IV**

| Diet                        | Weight gaina         |
|-----------------------------|----------------------|
|                             | 7 days | 14 days | 21 days |
| RS-610 (low tannin sorghum) | 2.0    | 14.5    | 22.7    |
| RS-610 + gelatin (4%)       | 7.1    | 20.1    | 28.3    |
| RS-610 + amino acid mix (4%)| 10.4   | 24.2    | 38.2    |
| RS-610 + Quebracho (4%)     | -20.0  | -24.8   | -28.7   |
| Savanna (high tannin sorghum)| -8.7  | -9.0    | -9.7    |
| Savanna + gelatin (1%)      | 4.5    | 16.0    | 22.8    |
| Savanna + amino acid mix (1%)| -6.6  | -1.6    | -3.0    |
| Savanna + Quebracho (1%)    | -19.0  | -26.3   | -31.0   |

a Weight gains are averages of six hamsters unless indicated.

**DISCUSSION**

Proline-rich proteins and glycoproteins are specifically synthesized and secreted by salivary glands of various animals. In addition to the hamster PRPs reported here, these unusual proteins have been isolated and characterized from human (see Ref. 2 for a review), rat (3, 41), mouse (5, 8), monkey (26), and rabbit (27). Mainly as a result of work from our laboratory it is now known that PRPs of mice, rats, and hamsters are tissue-specific products of multigene families which are dramatically induced by isoproterenol. These unusual proteins pose a number of interesting problems concerning gene structure and evolution, regulation of gene expression, post-translational modifications, and protein conformation.

Ann et al. (11) reported the complete sequence of hamster PRP gene H29. The amino acid composition of the derived polypeptide encoded by H29 (Fig. 9) is compared to those of HP43a and HP43b (Table II). The derived sequence of one reading frame from exon II of H29 is Ala-Thr-Ile-Tyr-Glu-Asp-Ser-Ile-Ser-Gln-Leu-Ser (11) which is identical to the N-terminal sequence of HP43a (Fig. 3) except in position 8 (aspartate instead of isoleucine) (Fig. 3). The amino acid sequence of HP43a is blank in position 9, and the derived sequence from H29 has a serine in this position. Therefore, this serine is likely phosphorylated or glycosylated. This same reading frame also encodes a polypeptide of 5 tandemly repeated peptides of amino acid sequence Pro-Pro-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Gly (Fig. 9 (11)). If HP43a is encoded by H29, it would contain 19 glutamates in 169 amino acids, which would explain why it is an excellent substrate for S. aureus V8 (Fig. 4, lane 2). From residues 53-63 (Fig. 9, Asp-Glu-Glu-Gly-Asp-Asp-Glu-Gly-Asp-Glu-Asp-Glu-Gly) nine out of eleven amino acids are acidic, identifying the polypeptide encoded by H29 as an acidic PRP. Based on the derived amino acid sequence, there is only one chymotrypsin site among 169 amino acids (P-45) which is consistent with the protease studies of HP43a (Fig. 4, lane 5). From the results obtained so far (composition analysis, acidic nature, N-terminal sequence analysis, and sensitivity to proteases), we concluded that H29 codes for HP43a or a very closely related protein.

Northern analysis (Fig. 7) showed two size classes of messages, 1100 and 850 bases, which is common to rat and mouse (6). The calculated size of hamster PRP mRNA encoded by H29 is 727 nucleotides plus the poly(A) tail. Possibly a message of 850 bases is encoded by H29. The wide range in sizes of induced PRPs (25-55 kDa, Fig. 6) cannot be explained by only two size classes of messages, and the anomalous behavior of PRPs on SDS-PAGE as demonstrated for translation products of rat PRP mRNAs (7) likely occurs with the hamster.

The hamster basic PRP HP45 was not phosphorylated (Table II), which is different from human basic PRP IB-I (24). Also there was no N-terminal homology of HP45 with other basic PRPs from rat, mouse, and human. On the other hand, N-terminal sequences among human, rat, and mouse acidic PRPs, and hamster acidic PRPs HP43a and HP43b are quite conserved (Fig. 3). As we reported previously (11), the evolution of the N-terminal region of acidic PRPs is apparently under negative selection against replacement substitution. In other words, the N-terminal regions of basic PRPs are under less stringent functional constraint than those of acidic PRPs. Recently, Braunlin et al. (28) reported that the N-terminal fragment of a human acidic PRP is involved in calcium binding, and this might explain the functional constraint.

With regard to regulation of PRP gene expressions, a different picture is presented by hamsters: (i) hamsters respond to isoproterenol treatment by an increased synthesis of PRPs (Figs. 1, 6, and 7), but there is little, if any, hypertrophic response (Table I) when compared to mice and rats; (ii) feeding tannins has essentially no effect on hamster salivary glands (Table III); (iii) hamsters fed a diet containing 2% tannin lose weight for about 3 days, as do rats (13) and mice (5), but then an unusual continued growth inhibition is ob-

![Fig. 3. Amino acid sequence derived from PRP gene H29. Repeat region arranged to align homologous sequences.](image-url)
served (Fig. 8). Hamsters maintained on 25% tannin diets failed to grow and even at 60 days were essentially at the same body weight as at 3 days. When the diets are switched, the experimental animals gained weight at almost the normal rate for young hamsters, while the control animals, now on a 25% tannin diet, lost about 20% of their weight. In about 20 days, both groups of hamsters were close to the same weight. Hamsters' PRPs have a similar affinity to tannin when compared to those of rat and mouse (Fig. 3) so this unusual growth inhibition cannot be explained by the failure of hamster PRPs to bind tannins. Clearly, the detrimental effects of tannins (see Ref. 29 for a review) were reversed by the induction of PRPs in rats (13) and mice (5). Hamsters are unusually susceptible to tannins, but this can be reversed by addition of gelatin to the diet (Table IV). Increasing the tannin content of the diet to 4% was fatal to hamsters with many animals dying within 3 days. There were no differences in the number of β-receptors and the activity of adenyl cyclase (Table V) between hamster and rat parotid glands, and there were several strong conservations at the nucleotide level of 5' flanking regions between hamster and mouse PRP genes (11), including cAMP-inducible elements.

Tannins ingested in large amounts, used as treatment for burns and as adjuvant for barium enemas, can cause circinomas, hepatotoxicity, and apparently other pathological and toxic problems (see Ref. 29 for a review). We believe that the PRPs in saliva constitute the first-line defense against tannins ingested. Dietary tannins and their induction and interaction with PRPs have recently been reviewed (29). A mechanism for regulation of the expression of the PRP multigene families by iso-proterenol and by tannins undoubtedly is to modulate levels of cAMP through activation of β-receptors in the parotid glands. The mode of inducing a β-agonist by dietary tannins is as yet unknown. Experiments using cell transfections with mouse and hamster PRP genes with different 5' upstream deletions have identified the nucleotide sequence inducible by cAMP, iso-proterenol, or forskolin as ATGTAAACAGTCA. The underlined G is missing in hamster PRP gene H29 (11). This sequence is missing from the 5' upstream region of the human PRP genes (12) and likely explains the induction of PRP genes in mice, rats, and hamsters and the constitutive nature in humans.

The response in the case of mice and rats or the lack of response in the case of hamsters to dietary tannins is likely modulated by events in the gastrointestinal tract. Addition of the β-blocker propranolol to the diet blocks the effects of tannins on the parotid glands of rats and mice (29). Data on β-receptors and adenylcyclase suggest that induction of PRPs in hamsters is modulated by β-agonists and β-receptors. Information on transfections with PRP genes MP2 (6) and H29 (11) and with gene deletions of the 5' upstream region of these genes clearly shows that cAMP is at least one causative agent. Both MP2 and H29 have almost identical upstream regulatory sequences so that the lack of regulation of expression of the hamster PRP multigene family by tannins apparently is not at the glanulard level. The unusual growth-inhibiting effects of hamsters by tannins do not result in stunting, at least not within 6 months of feeding, since these animals responded to a change in diet by exhibiting essentially normal growth. Effects on hormones regulating growth patterns need to be investigated.

Acknowledgments—We wish to thank Drs. Mark Hermodson and Scott Huckle for performing the amino acid sequencing and Dr. Tom Asquith for performing the tannin binding studies.

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P. S. Wright, D. K. Ann, and D. M. Carlson, unpublished observations.