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Strain-specific differences in the proline-rich proteins and glycoproteins induced in rat salivary glands by chronic isoprenaline treatment

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Parotid and submandibular glands were isolated from five strains of rat after chronic injection of the β-adrenergic receptor agonist isoprenaline (isoproterenol). The glands were observed to have undergone a marked increase in wet weight, owing to hypertrophy and hyperplasia. The 100000*g* soluble fraction of gland cell lysates were extracted with 10% (w/v) trichloroacetic acid, and the soluble material subsequently analysed by SDS (sodium dodecyl sulphate)/polyacrylamide-gel electrophoresis. By this procedure, evidence was obtained for the induction, in isoprenaline-treated parotid and submandibular glands, of proline-rich proteins with apparent M_r values ranging from 20000 to 40000. Heterogeneity was evident in the proteins produced for a specific gland between the rat strains, although the amino acid compositions were the same. Products from induced mRNAs translated *in vitro* had similar mobilities in SDS/polyacrylamide gels, despite the apparent difference in mobility of trichloracetic acid-extracted proline-rich proteins from the various strains. Strain-specific differences were noted for the proline-rich glycoproteins from control salivary glands as well as those induced as a consequence of isoprenaline treatment. Although the glycoproteins had similar amino acid compositions, there was considerable heterogeneity in the carbohydrate compositions for these proteins, suggesting that the differences were the result of post-translational modifications during glycosylation. Induction of the increased activity of the Golgi membrane marker enzyme UDP-galactose : 2-acetamido-2-deoxy-D-glucosamine 4β-galactosyltransferase (EC 2.4.1.22) occurred to the same extent in the parotid glands of all strains examined. There was no change in the specific activity of a second enzyme, UDP-galactose : *N*-acetylgalactosaminyl-protein 3β-galactosyltransferase (no EC designation).

The salivary glands from several mammalian species have been shown to produce and secrete an unusual set of proteins composed primarily of three amino acids (proline, glycine and glutamic acid), which represent approx. 80% of the total residues (Mandel *et al.*, 1965; Levine *et al.*, 1969; Bennick & Connell, 1971; Oppenheim *et al.*, 1971; Muenzer *et al.*, 1979*b*). To date, the human system has been the most extensively studied. The PRPs from human parotid and submandibular saliva can be divided into acidic, basic and glycosylated proteins (Isemura *et al.*, 1980, 1982; Porchet *et al.*, 1983; Saitoh *et al.*, 1983*a,b,c*). Similarities in *N*-terminal sequences of the basic PRPs has led to the suggestion that these peptides are the result of individual genes derived from a common ancestral gene or arise from proteolytic fragmentation of a precursor polypeptide (Saitoh *et al.*, 1983*c*).

Biochemical analysis of the 100000*g* cell lysate supernatant from rat parotid and submandibular glands, after chronic treatment with the β-adrenergic receptor agonist, isoprenaline, have revealed specific alterations in the pattern of protein biosynthesis. This treatment has been shown to induce the biosynthesis *de novo* of a series of basic PRPs in both glands in strains of Wistar rats (Muenzer *et al.*, 1979*a,b*; Mehancho & Carlson, 1983; Humphreys-Beher, 1984*a*). Untreated parotid glands normally produce a single proline-rich glycoprotein of 200 kDa, but on chronic injection of isoprenaline a second glycoprotein is induced (Humphreys-Beher, 1984*a*). In the case of the

Abbreviations used: PRPs, proline-rich proteins; SDS, sodium dodecyl sulphate; poly(A)$^+$, polyadenylylated.

submandibular gland, two glycoproteins of 180 and 200kDa are present in control animals (Humphreys-Beher *et al.*, 1982; Humphreys-Beher, 1984*a*). However, after administration of isoprenaline, a new glycoprotein is produced with a molecular mass of 190kDa (Humphreys-Beher, 1984*a*). The parotid gland additionally shows a 6–10-fold increase in the Golgi membrane marker enzyme UDP-galactose:*N*-acetylglucosamine 4β-galactosyltransferase (Humphreys-Beher, 1984*b*).

In the present study the salivary glands from five strains of rat were analysed for the synthesis of basic PRPs and proline-rich glycoproteins after chronic administration of isoprenaline. Strain-specific differences appear to be present in the PRPs synthesized in the parotid and submandibular glands examined after treatment. Although the proteins showed differences in mobility in SDS/polyacrylamide gels, analysis of their amino acid composition showed them to be similar in composition. Similarities in products of translation *in vitro* suggest that the differences in trichloroacetic acid-isolated samples are the result of strain-specific patterns of post-translational processing of proteins. The same results were obtained with the proline-rich glycoproteins. The different strains produced various combinations of the 190, 180, 200 and 220kDa glycoproteins before and after chronic treatment with isoprenaline. Although each of these proteins had similar amino acid compositions, they appear to differ from each other in the post-translational processing of the carbohydrate side chains. Induction of the increased 4β-galactosyltransferase activity was the same in all cases examined.

Experimental procedures

Materials

(±)-Isoprenaline hydrochloride, bovine submaxillary mucin, 2-thiobarbituric acid and caesium chloride were from Sigma Chemical Co. UDP-[1-^{14}C]galactose (sp. radioactivity 300mCi/mmol) and [^{35}S]methionine (sp. radioactivity 800Ci/mmol) were purchased through New England Nuclear Corp. and Amersham respectively. All reagents used for polyacrylamide-gel electrophoresis were purchased from Bio-Rad. Reticulocyte lysates for translation *in vitro* were obtained from Bethesda Research Laboratories. All other chemicals were of analytical grade and obtained through commercial sources.

Isoprenaline treatment

Male rats weighing 175–225g received intraperitoneal injections of 5mg of (±)-isoprenaline hydrochloride dissolved in 0.5ml of 150mM-NaCl. Unless otherwise stated, animals were injected daily for 10 days and were fed *ad libitum*. Rat strains employed in this study were Wistar and Long-Evans (obtained through the above sources), Lewis, SHR/NCr1BR (spontaneously hypertensive) and WKY/NCr1BR (normotensive controls) purchased from Charles River Breeding Laboratories, Wilmington, MA, U.S.A. Long-Evans strain rats were generously given by Dr. Gopal Das, Department of Biological Sciences, Purdue University, Lafayette, IN, U.S.A., and Dr. Charlotte Schneyer, Department of Physiology and Biophysics, University of Alabama at Birmingham.

Tissue preparation and protein isolation

Parotid and submandibular glands were identified by gross morphology. The glands were removed from animals after being anaesthetized with sodium pentobarbital and killed by exsanguination. The soluble and insoluble membrane fractions were prepared by homogenization in 10mM-Tris/HCl buffer, pH8.0, with a Dounce apparatus. The slurry was then centrifuged at 100000***g*** for 1h. Protein assays were performed by a modification of the Lowry method, with bovine serum albumin (Sigma Chemical Co.) as a standard (Schacterle & Pollack, 1973). Purification of the salivary-gland glycoproteins and PRPs was by extraction in 10% trichloroacetic acid as described previously (Mehancho & Carlson, 1983; Humphreys-Beher, 1984*a*). The insoluble proteins were removed by centrifugation at 15000***g*** for 15min and trichloroacetic acid removed by dialysis against 10% acetic acid followed by freeze-drying, instead of diethyl ether extraction.

Polyacrylamide-gel electrophoresis and protein staining

Protein samples were subjected to electrophoresis in 0.75mm-thick 10%-(w/v)-polyacrylamide gel by using the modified Tris/glycine Laemmli system described by Pugsley & Schnaitman (1979). All gels were fixed and stained by a modification of the method of Fairbanks *et al.* (1971) as described elsewhere (Humphreys-Beher & Wells, 1984). Samples for gels were made up to 1 mg of protein/ml of sample buffer. A 35μg sample of protein per well was routinely used in subsequent gel electrophoresis. Proline-rich glycoproteins were stained for carbohydrate by using the periodate/Schiff-reagent staining procedure of Fairbanks *et al.* (1971) without modification.

Protein purification

Trichloroacetic acid-soluble glycoproteins were isolated from the PRPs by chromatography on a column (1cm×90cm) of Sephadex G-100 (Pharmacia). The protein was eluted with 10mM-Tris buffer, pH7.5, and 2ml fractions were

collected. Protein elution was monitored by A_{230} as described by Henkin & Wolf (1975) and Mehancho & Carlson (1983). Proline-rich proteins were isolated by preparative polyacrylamide-gel electrophoresis (3.0mm thickness) after identification of the protein in the gel and subsequent electroelution.

Amino acid analysis

Purified PRPs and proline-rich glycoproteins were dialysed against 10% acetic acid to remove Tris buffer, and then freeze-dried. Portions (0.2mg) of protein were analysed for amino acid composition by hydrolysis in 6M-HCl for 18h as described previously (Muenzer *et al.*, 1979*a*,*b*). Amino acid analyses were performed on a Beckman 119 analyser by using single-column methodology. Amino acid sequencing was performed on Pronase-generated glycopeptides from parotid glycoprotein on an Applied Biosystems model 470A protein sequencer.

Carbohydrate analysis

Neutral sugars and amino sugars were assayed by gas chromatography of the alditol acetates prepared as described by Albersheim *et al.* (1967). The alditol acetate derivatives of the glycoproteins were chromatographed on a Varian model 3700 gas chromatograph equipped with a CDC111 integrator and chart recorder. A 25-metre SP2100 capillary column was used and the run was programmed for 12min at 160°C, increased at 2°C/min up to 250°C, and held constant for 10min. The injector temperature was set at 250°C and the detector temperature at 320°C. Sialic acid was assayed by the thiobarbituric acid procedure of Warren (1959), both before and after hydrolysis (0.1M-H_2SO_4, 80°C, 1h) and neuraminidase (Sigma).

Galactosyltransferase assay

The activities of UDP-galactose:*N*-acetylglucosamine 4β-galactosyltransferase and UDP-galactose:*N*-acetylgalactosaminyl-protein 3β-galactosyltransferase were measured by a modification of the assay described by Carlson *et al.* (1973). Membrane fractions were obtained as described above and resuspended in 10mM-Tris/HCl buffer, pH8.0, to give a final protein concentration of 200μg/ml. Specific activities of the galactosyltransferases are expressed as nmol/min per mg of protein. The assay of radioactive products was performed as described previously by Humphreys-Beher (1984*b*).

Isolation of poly(A)$^+$ RNA

Total gland RNA was isolated by the method of Chirgwin *et al.* (1979). Immediately on removal, 1g of fresh tissue was homogenized in 4M-guanidine thiocyanate, pH7.0, containing 1.0% sodium sarkosyl, 50mM-EDTA and 25mM sodium citrate. The RNA was subsequently recovered by an 18h centrifugation through 5.7M-CsCl at 35000rev./min in an SW27 rotor. Poly(A)$^+$ RNA was obtained from total RNA by using the procedure of Aviv & Leder (1972) with oligo(dT)–cellulose.

Translations of RNA in vitro

Poly(A)$^+$ RNA was translated *in vitro* by using the method of Pelham & Jackson (1976) with a reticulocyte lysate (nuclease-treated) prepared by Bethesda Research Laboratories. The reaction volumes used were 60μl containing 0.5μg of normal or isoprenaline-treated parotid or submandibular-gland RNA. Incorporation of [^{35}S]methionine into protein was determined by 10% trichloroacetic acid precipitation of 5μl from translations *in vitro* on glass filters. Samples from translations were prepared for electrophoresis in SDS/polyacrylamide gels and subsequent X-ray fluorography as described by Humphreys-Beher (1984*b*).

Methylation analysis

For methylation analysis the purified glycoproteins from the isoprenaline-treated WKY/NCr1BR parotid gland (200kDa) and the glycoprotein from the isoprenaline-treated Long-Evans strain (220kDa) were used. Glycopeptides were isolated after exhaustive Pronase (Sigma) digestion, followed by chromatography on Sephadex G-25 using 10mM-sodium phosphate buffer, pH7.4, and desalting on Bio-Gel P-4 by the procedure of Mehancho & Carlson (1983). Permethylation was performed on 1mg of the glycopeptide by the method of Hakomori (1964). A 1ml portion of 3M-potassium dimethylsulphonyl anion was added to each tube and mixed with the sonicated samples for an additional 4h.

The oligosaccharides were then methylated by the addition of 0.5ml of methyl iodide and stirred overnight. This was repeated a second time to ensure complete methylation of the amino sugars. Methylated oligosaccharides were recovered by chromatography on Sep-paks-C18 (Waters), with methanol as the extraction solvent.

Preparation of alditol acetates from the methylated oligosaccharides was performed by the method of Albersheim *et al.* (1967). Samples in 0.5ml of 2.5M-trifluoroacetic acid containing 100nM-inositol as an internal standard were autoclaved for 90min. Reduction of samples was performed by the addition of 1ml of 1M-NH_3, containing 2mg of $NaBH_4$/ml, for 24h. This mixture was then neutralized with 250μl of acetic acid and evaporated at 35°C. The samples were

extracted with 1.5ml of h.p.l.c.-grade methanol (Mallinckrodt). Subsequently the samples were resuspended in 200μl of acetic anhydride and again autoclaved for 90min. Methylation analysis was carried out on a Hewlett–Packard HP5985A GC/MS/DS system. Samples were chromatographed on a 25-metre HP-SE-54 capillary column using a splitless injection of a 1μl sample volume as described by Bjorndal *et al.* (1967, 1970) and by Stellner *et al.* (1973). Chromatographic conditions were isothermal at 60°C for 5min and the temperature increase programmed at 5°C/min to 220°C. The mass spectrometer was operated in the electron-impact mode (70eV, source and analysis temperature, 200°C; injector temperature 300°C). Two chromatographic determinations were made on each of three separate derivatives of the 200 and 220kDa glycoproteins.

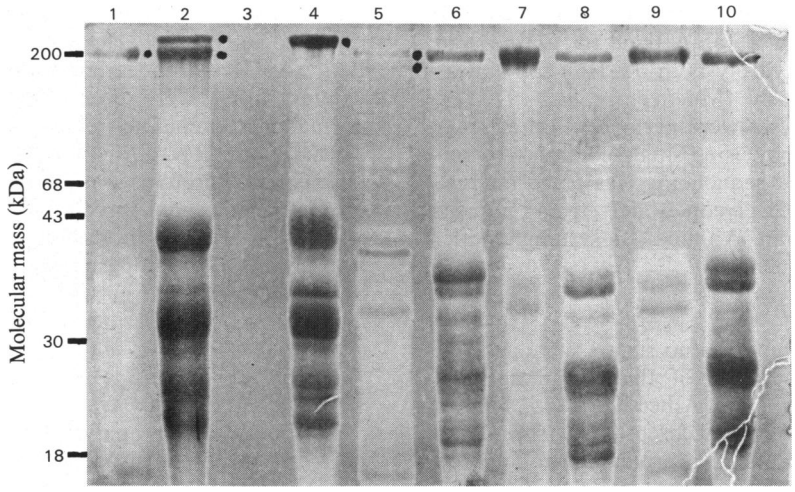

Fig. 1. *10%-(w/v)-Polyacrylamide-gel electrophoresis of parotid-gland trichloroacetic acid extracts*
Lane 1, Wistar-strain, control; lane 2, Wistar strain, isoprenaline-treated; lane 3, Long-Evans strain, control; lane 4, Long-Evans strain, isoprenaline-treated; lane 5, Lewis strain, control; lane 6, Lewis strain, isoprenaline-treated; lane 7, SHR/NCr1BR strain, control; lane 8, SHR/NCr1BR, isoprenaline-treated; lane 9, WKY/NCr1BR strain, control; lane 10, WKY/NCr1BR, isoprenaline-treated. About 35μg of protein was run per well. The gels were subsequently stained with Coomassie Brilliant Blue R-250 after electrophoresis. Molecular-mass standards were: bovine serum albumin, 68kDa; ovalbumin, 43kDa; trypsinogen, 24kDa; and β-lactoglobulin, 18kDa. The bold dots indicate the high-molecular-mass proline-rich glycoproteins.

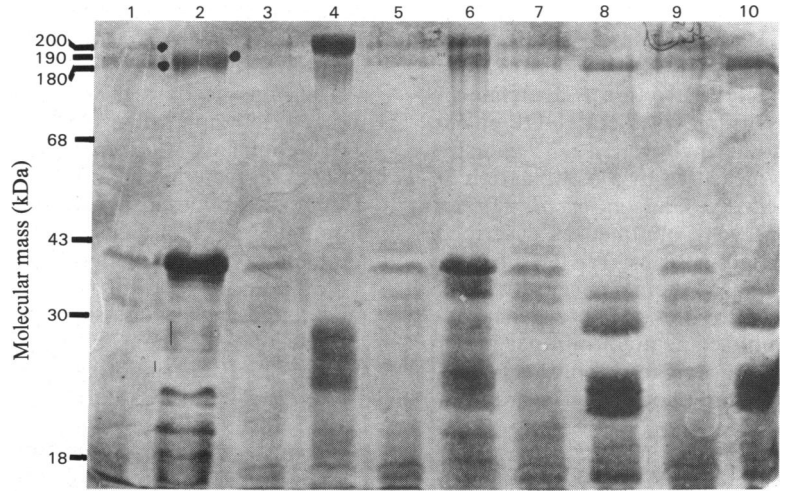

Fig. 2. *10%-(w/v)-Polyacrylamide-gel electrophoresis of submandibular-gland trichloroacetic acid extracts*
Lanes are the same as those in Fig. 1.

Results and discussion

The soluble cell fractions from parotid and submandibular-gland homogenates were extracted with 10% trichloroacetic acid before analysis on SDS/polyacrylamide gels. As shown in Figs. 1 and 2, isoprenaline treatment had the same effect on the induction of PRP biosynthesis in the parotid and submandibular glands from the different rat strains as it had on Wistar rats. However, there were differences in the number as well as the relative mobility of the PRPs from the different strains. The relative amounts of the two bands present in trichloroacetic acid extracts of isoprenaline-treated parotid glands from Lewis, SHR/NCr1BR and WKY/NCr1BR strains suggest possible differences in transcriptional or translational processing of the mRNA for these two proteins between these strains (Fig. 1, lanes 6, 8 and 10). Differences in the gel mobilities of trichloroacetic acid-extracted PRPs from the isoprenaline-treated submandibular glands of the various rat strains were also observed after polyacrylamide-gel electrophoresis (Fig. 2, lanes 2, 4, 6, 8 and 10).

The mRNA was isolated from the parotid and submandibular glands of several of these strains to determine if differences in transcriptional or translational processes were responsible for the variance of strain-specific mobilities of the PRPs observed in SDS/polyacrylamide gels. Translation *in vitro* of mRNA from parotid glands of control and isoprenaline-treated animals gave the same patterns of induced PRP-mRNA translation products in SDS/polyacrylamide gels (Fig. 3). This is in contrast with the differences observed in Fig. 1, indicating strain-specific differences in the rates of specific mRNA translation and proteolytic processing. Translation products for the mRNA from the submandibular glands gave identical patterns for the strains (not shown), although the trichloroacetic acid-extracted submandibular PRPs have very different mobilities (Fig. 2).

PRPs were isolated after preparative gel electrophoresis as described by Mehancho & Carlson (1983) and analysed for their amino acid composition. An example of the protein purification is indicated in Fig. 4 (inset, lane *d*). The compositions for the PRPs isolated from the different salivary glands were similar to each other (Table 1). These proteins were all unique in the complete absence of aromatic and sulphur-containing amino acids. The amino acid compositions were similar not only for the PRPs from the various strains, but also of those induced in the parotid and submandibular glands in the same strain.

The high M_r PRPs in the different strains studied were identified as glycoproteins after staining for carbohydrate by staining with periodic acid/Schiff reagent (Humphreys-Beher, 1984*a*) (results not shown). Control and isoprenaline-treated parotid glands show the synthesis of a number of glycoproteins with M_r 180000, 200000 and 220000 (Fig. 1). The untreated Long-Evans strain did not produce a glycoprotein.

The untreated control submandibular glands from the various strains characteristically produced two glycoproteins with apparent M_r values of 180000 and 200000 previously reported to be present in the Wistar strain (Humphreys-Beher, 1984*a*) (Fig. 2, lanes 1, 3, 5, 7 and 9). After isoprenaline treatment, distinct differences were found in the mobility of the glycoproteins produced by the different strains, as shown in Fig. 2.

The glycoproteins from the trichloroacetic acid-

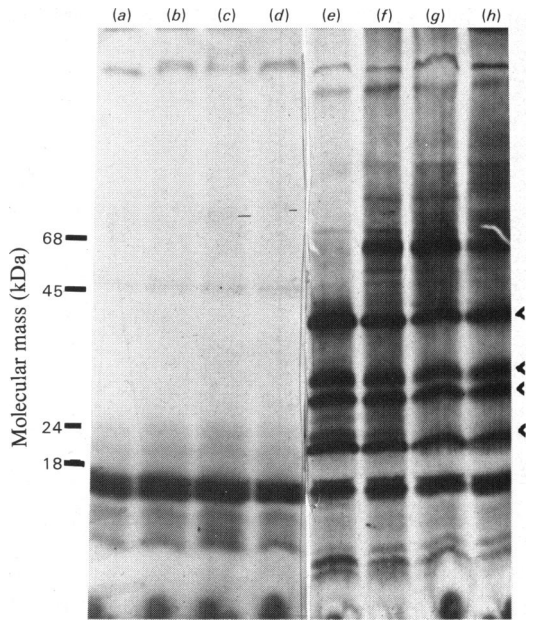

Fig. 3. *X-ray fluorogram of translations in vitro from strain-specific mRNA*

Poly(A)$^+$ mRNA was translated *in vitro* in a (Bethesda Research Laboratories) system with 0.75 μg of mRNA and radiolabelled with [^{35}S]methionine. A 45000 c.p.m. portion of each translation was applied per well and electrophoresed on an SDS/10%-(w/v)-polyacrylamide gel. Gels were dried on filter-paper backing after impregnation with En^{3}Hance (Amersham) and exposed at -70°C for 48 h. (*a*) Untreated Wistar parotid gland; (*b*) translation of untreated mRNA from Long-Evans strain; (*c*) translation of untreated Lewis-strain mRNA; (*d*) translation of SHR/NCr1Br parotid-gland mRNA. Isoprenaline-treated mRNA from parotid glands listed in respectively. Arrowheads (<) indicate mobility of induced PRPs.

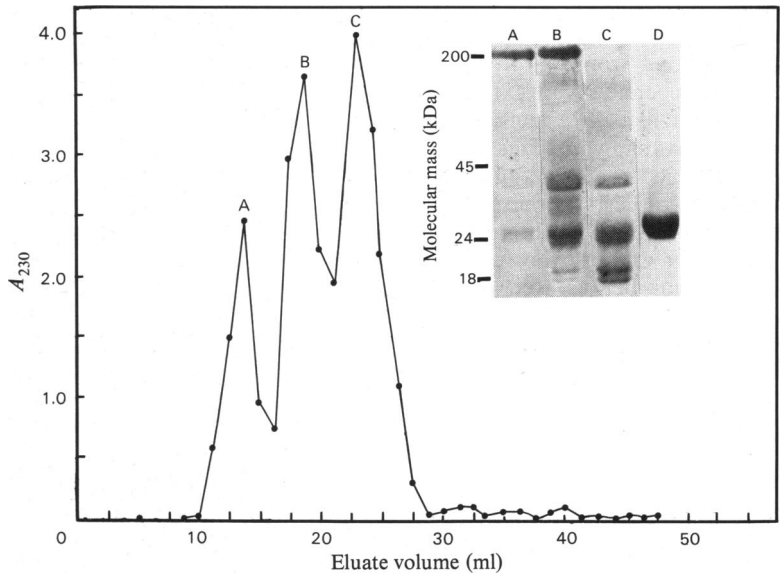

Fig. 4. *Sephadex G-100 chromatography*

Parotid- and submandibular-gland trichloroacetic acid extracts were treated as described in the Experimental procedures section. Flow rates were maintained at 15ml/h per cm^2 and 2ml fractions were collected. The column eluate was monitored at 230nm. Peaks were pooled and run on polyacrylamide gels. The inset gels show the profiles of proteins contained in peaks A, B and C respectively. The sample shown here is the WKY/NCr1Br parotid treated with 5mg of isoprenaline/200g body wt. of animal. Inset lane D shows the purified 26kDa PRP purified by preparative SDS/polyacrylamide-gel electrophoresis and re-electrophoresed to show the isolation of a single band.

Table 1. *Amino acid analysis of parotid- and submandibular-gland PRPs*

(*a*) Parotid gland

Amino acid / Strain ... / PRP size (kDa) ...	Content (mol/100mol)				
	Wistar 40	Long-Evans 40	Lewis 36	SHR/NCr1BR 35	WKY/NCr1BR 26
Aspartic acid	3.1	3.0	3.0	2.9	3.0
Threonine	0.6	0.0	0.2	0.2	0.0
Serine	2.1	2.3	2.2	2.0	2.1
Glutamic acid	22.5	22.9	22.3	22.6	22.3
Proline	42.0	41.3	42.1	41.9	42.0
Glycine	23.2	22.6	22.7	23.0	22.9
Alanine	0.0	0.0	0.1	0.0	0.2
Lysine	1.3	1.0	0.7	1.0	1.0
Arginine	5.1	5.0	4.7	4.8	4.9

(*b*) Submandibular gland

Amino acid / Strain ... / PRP size (kDa) ...	Content (mol/100mol)				
	Wistar 40	Long-Evans 28	Lewis 40	SHR/NCr1BR 30	WKY/NCr1BR 30
Aspartic acid	3.0	2.9	3.0	3.0	3.0
Threonine	0.0	0.2	0.1	0.1	0.1
Serine	2.4	2.6	2.4	2.4	2.5
Glutamic acid	22.7	23.0	22.9	22.5	22.9
Proline	41.8	42.1	42.0	42.0	42.0
Glycine	22.9	23.1	23.0	22.9	22.0
Alanine	0.9	0.0	0.0	0.0	0.0
Lysine	0.8	1.2	1.0	1.0	1.1
Arginine	5.0	4.8	4.8	5.0	4.9

extracted PRP mixture of the various rat strains were purified singularly or in combination by chromatography on a column of Sephadex G-100. As shown in Fig. 4, three peaks were routinely eluted from the column. Typically, pure proline-rich glycoproteins were isolated in the column void volume. Amino acid analysis and carbohydrate analysis of alditol acetate derivatives were subsequently performed on the purified proteins. The proline-rich glycoproteins were not as 'proline-rich' as the non-glycosylated PRPs (Table 2). These glycoproteins were shown to have, in addition to proline, particularly high amounts of glycine and glutamic acid and typically lacked aromatic and sulphur-containing amino acids. Although the proteins showed differences in mobility on SDS/polyacrylamide gels, their overall amino acid compositions were very similar.

Analysis of the carbohydrate composition revealed major differences between the various glycoproteins produced in rat parotid and submandibular glands. The most significant difference in these proteins was the lack of sialic acid in the parotid-gland glycoproteins (Table 3). The 220kDa glycoprotein had a two- to three-residue increase in the amount of galactose over that present in the 200kDa glycoprotein, as well as a possible single-residue difference in *N*-acetylglucosamine, when the values are expressed relative to the standard three-mannose core structure of *N*-linked oligosaccharides. The other observed difference for these proteins was the varying amounts of total carbohydrate (Table 3).

Since the parotid gland treated with isoprenaline generally produced the 200 or 220kDa glycoproteins that appear to differ in the amount of galactose and *N*-acetylglucosamine, it was of particular interest to determine and compare the structures of their respective oligosaccharide side chain for similarities. The two glycoproteins from isoprenaline-treated Long-Evans strain and isoprenaline-treated WKY/NCr1BR parotid glands were analysed by g.l.c.–m.s. to obtain this information. Figs. 5(*a*) and 5(*b*) show the g.l.c. elution profile of permethylated alditol acetates of the carbohydrates from the 200 and 220kDa glycoproteins respectively. The major difference in the profile is the increase in the peak at 15.93min in the chromatogram for Fig. 5(*b*) (220kDa glycoprotein). The electron-mass profile of this peak identified terminally linked galactose, whereas the profile of the peak at 18.85min showed a decrease in terminally linked *N*-acetylglucosamine when compared with Fig. 5(*a*). The electron-mass profiles of peaks at 14.4, 16.7, 17.7, 18.1, 19.7 and 20.75min identified terminal fucose, 2-linked mannose, 2,4-linked mannose, 3,6-linked mannose, 4-linked *N*-acetylglucosamine and 4,6-linked *N*-acetylglucosamine respectively. From a determination of the ratio of the sugar peaks in the chromotograms of Fig. 5, the oligosaccharide structures for the 200 and 220kDa glycoproteins

Table 2. *Amino acid analysis of parotid- and submandibular-gland proline-rich glycoproteins*

Amino acid / Glycoprotein size (kDa) ...	Content (mol/100mol)					
	200*	180†	220‡	190§	180/200‖	200/220¶
Aspartic acid	14.5	14.3	14.6	14.1	14.1	14.3
Threonine	7.4	6.8	6.8	7.1	7.0	7.4
Serine	1.5	1.1	1.1	1.2	1.2	1.3
Glutamic acid	21.3	22.0	21.7	22.1	22.0	21.8
Proline	25.8	42.2	24.9	25.0	24.9	25.5
Glycine	11.1	12.0	11.9	10.9	11.4	11.6
Alanine	0.6	1.2	1.3	0.5	1.0	1.0
Valine	0.3	0.3	0.2	0.4	0.2	0.2
Methionine	0.2	0.2	0.0	0.1	0.2	0.0
Isoleucine	0.6	0.4	0.3	0.3	0.6	0.4
Leucine	1.2	0.4	1.2	1.2	1.2	1.1
Histidine	8.1	8.1	8.8	8.2	7.9	8.4
Lysine	2.0	3.0	2.9	2.7	2.8	1.9
Arginine	5.5	5.8	5.3	5.8	6.3	6.5
Phenylalanine	0.1	0.1	0.2	0.1	0.1	0.1

* Control Wistar parotid gland.
† Treated SHR/NCr1BR submandibular gland.
‡ Treated Long-Evans parotid gland.
§ Treated Wistar submandibular gland.
‖ Control Lewis parotid gland.
¶ Treated Wistar parotid gland.

Table 3. *Carbohydrate composition of parotid- and submandibular-gland proline-rich glycoproteins*
Sources of the glycoproteins are as listed in Table 2.

Sugar	Glycoprotein size (kDa) ...	Glycoprotein content (no. of residues, with mannose ≡ 3)					
		200	180	220	190	180/200	200/220
Fucose		1.2	0.7	0.9	0.9	1.0	1.5
Mannose		3.0	3.0	3.0	3.0	3.0	3.0
Galactose		1.3	2.0	3.4	1.9	2.5	8.0
N-Acetylglucosamine		4.7	3.8	6.5	3.7	4.3	7.2
Sialic acid		0.0	2.1	0.0	1.8	1.2	0.0
	Carbohydrate (%) ...	40	38	45	35	40	48

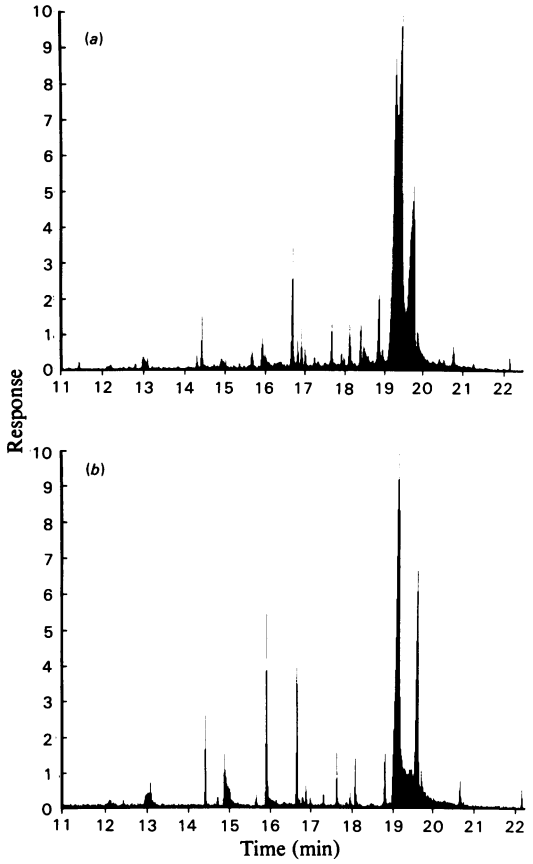

Fig. 5. *Gas chromatogram of permethylated alditol acetates derived from (a) WKY/NCr1BR-strain parotid-gland 200kDa glycoprotein and (b) Long-Evans-strain parotid-gland 220kDa glycoprotein*
Samples were chromatographed on a Hewlett-Packard HP5985A equipped with a 25-metre HP-SE-54 capillary column as described in the Experimental procedures section. The internal standard, inositol, was eluted at 19.0–19.2min.

shown in Fig. 6 were proposed. The core structure of both oligosaccharides is a triantennary structure, terminating in *N*-acetylglucosamine for the 200kDa protein, and in galactose with an additional *N*-acetylglucosamine for the 220kDa glycoprotein. The terminal galactose as well as terminal *N*-acetylglucosamine may be on any of the three branches of the oligosaccharide side chain. The peak at 14.9min on Fig. 5(*b*) could not be identified from its electron mass spectrum as a sugar. The peak at 19.2min was identified as the internal standard, inositol.

Parotid glands from Wistar rats have been shown, after isoprenaline treatment, to demonstrate an increased specific activity for the Golgi membrane marker enzyme UDP-galactose:*N*-acetylglucosamine 4β-galactosyltransferase (Humphreys-Beher, 1984*b*). The membrane fraction isolated from the parotid gland of the different strains showed a characteristic induction in each case similar to the Wistar parotid glands. The specific activity normally present ranged from 0.21 to 0.32nmol of galactose/min per mg of protein, whereas values of 1.68–1.93nmol of galactose/min per mg of protein were routinely obtained after chronic isoprenaline treatment. A second enzyme activity, UDP-galactose:*N*-acetylgalactosaminyl-protein 3β-galactosyltransferase, remained unchanged in treated parotid glands. Neither enzyme activity was affected by chronic isoprenaline treatment in any of the submandibular glands examined in the present study (results not shown).

In the present study, the parotid and submandibular glands from various strains of rat were examined for the induction of PRPs and proline-rich-glycoprotein biosynthesis as a consequence of chronic isoprenaline treatment. The previously reported biochemical changes in the salivary-gland cell lysates were found to take place in each strain. Major differences were established for the relative mobilities in SDS/polyacrylamide gels for the PRPs and glycoproteins.

The similarity in the amino acid composition for the PRPs suggests the existence of a PRP gene family. A comparison of products of mRNA from the parotid glands translated *in vitro* would indicate that diversity in this class of proteins was

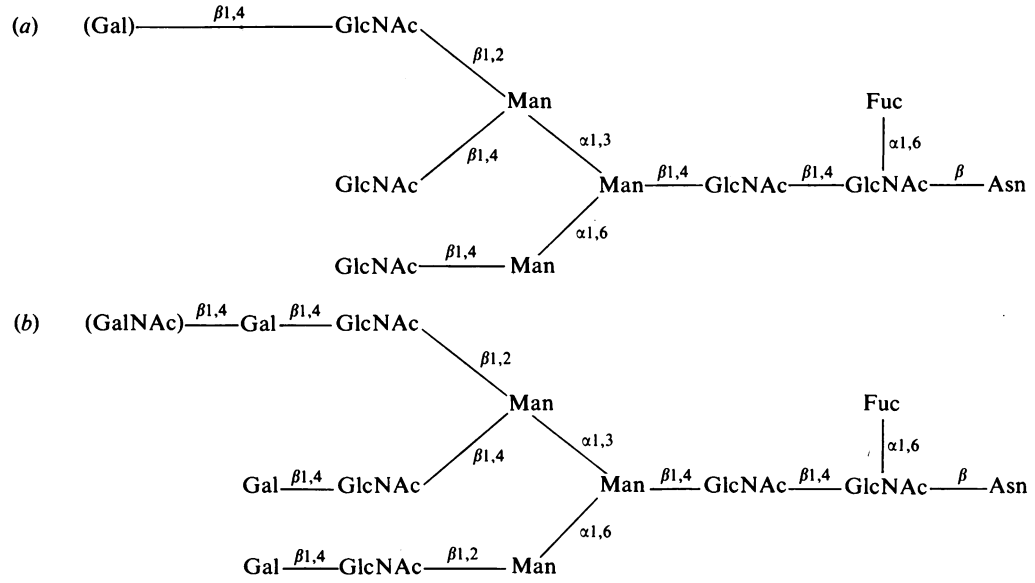

Fig. 6. *Proposed oligosaccharide structures determined from the g.l.c.–m.s. profile for (a) WKY/NCr1BR parotid-gland 200kDa glycoprotein and (b) 220kDa glycoprotein isolated from treated Long-Evans parotid glands*

generated by differences in translation rates of the mRNA and by post-translational processing. The most likely mechanism for this would be proteolytic processing similar to that observed for the human PRPs (Saitoh *et al.*, 1983*a,b,c*; Wong *et al.*, 1983). Further attempts to characterize these proteins by proteolytic generation of peptide maps were unsuccessful. Although specific salivary-gland proteinases exist for processing these types of proteins (Wong *et al.*, 1983), typically used proteinases did not affect these proteins. This could be accounted for by the absence of specific amino acids commonly recognized by these proteinases (i.e. thermolysin, specific for aromatic amino acids) and the high level of proline, which can interfere with cleavage at certain amino acid residues. Characterizing the proteins by *N*-terminal sequencing established that all the PRPs and glycoproteins isolated for study were blocked at the first amino acid in each instance.

The different amino acid compositions of the proline-rich glycoproteins indicated the presence of a second family of proteins closely related to the basic non-glycosylated PRPs. Strain-specific differences in the mobility of this class of proteins was apparently due in part to the differences in the composition and amounts of the oligosaccharide side chains. Isoelectric focusing of parotid and submandibular-gland glycoproteins that had had sialic acid removed all showed a similar isoelectric point (8.5), a value previously identified for the 200 and 220kDa glycoproteins from the Wistar rat (Humphreys-Beher & Carlson, 1982). Amino acid sequence analysis of proline-rich glycopeptides from the parotid and submandibular glands of Wistar-strain rats has shown that these proteins have the same peptide sequences, although they appear to differ in polysaccharide composition (Humphreys-Beher & Carlson, 1982; Mehancho & Carlson, 1983; Uhlig *et al.*, 1984). Sequence analysis for the Long-Evans- and WKY/NCr1BR-strain parotid glycopeptide utilized in the above studies show the same first five amino acids as the Wistar-strain glycoproteins. This sequence was Asp-Gly-Asn-Gln-Thr.

Although differences in glycosylation could account for the changes in the M_r values for these proteins, it is still rather unclear to what extent carbohydrate side-chain structures or their composition affects SDS binding and polyacrylamide-gel mobility in determining apparent M_r (Segrest & Jackson, 1972). The unique amino acid composition of the PRPs is in itself sufficient to give aberrant mobility in SDS/polyacrylamide gels (Muenzer *et al.*, 1979*b*).

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