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# INHIBITION OF GASTROINTESTINAL MUCOSAL GLYCOPROTEIN SYNTHESIS BY THE β-ADRENERGIC BLOCKING DRUG, PRACTOLOL

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**Abstract**—The effect of administration of practolol and other β-blocking agents on gastrointestinal mucosal glycoprotein synthesis was studied in the rat. Practolol, at a dose of 50 mg/kg, inhibited the incorporation of *N*-acetylglucosamine into gastric mucosal glycoproteins, while acebutolol, atenolol, pronethalol and propranolol had no inhibitory effect, even at a dose of 200 mg/kg. In addition, practolol inhibited the incorporation of *N*-acetylneuraminic acid, D-fucose and L-serine into gastric mucosal glycoproteins, while the other β-blocking agents had no effect. Administration of practolol caused no significant change in the rate of incorporation of glycoprotein precursors into intestinal mucosal glycoproteins. These results indicate that of the β-blocking drugs studied, inhibition of glycoprotein synthesis is associated only with practolol and is independent of its β-blocking effect.

Practolol was the first $\beta_1$-adrenergic blocking agent to be used in the successful treatment of hypertension [1] but was subsequently withdrawn following the reporting of adverse side-effects. Long-term administration of practolol in a small minority of patients leads to ulceration of the eye, nasal and oral mucosae, a condition described as the oculomucocutaneous syndrome [2–6] and possibly involving mucus production [7]. A similar syndrome, but less severe and occurring less frequently, has also been associated with long-term ingestion of other β-blocking drugs [8, 9]. Practolol administration has also been found to give rise to sclerosing peritonitis associated with intestinal adhesion and obstruction [10], indicating that the drug may interfere with peritoneal mucus production.

It is not yet known whether these adverse effects of practolol are specific to this drug or form an extension of its pharmacological effect, being an ultimate consequence of β-blockade. In the present study the effect of practolol on the synthesis of gastrointestinal mucosal glycoproteins [11], as a model for ocular and peritoneal mucus production, is investigated and compared to that of other β-blocking agents.

## MATERIALS AND METHODS

*N*-Acetyl-D-[1-$^3$H] glucosamine (sp. act. 3.0–4.7 Ci/mmole), D-[1-$^3$H] fucose (sp. act. 3.9 Ci/mmole), *N*-acetyl [4, 5, 6, 7, 8, 9-$^{14}$C] neuraminic acid (sp. act. 0.245 Ci/mmole) were purchased from the Radiochemical Centre (Amersham, U.K.) and L-[G-$^3$H] serine (sp. act. 1.8 Ci/mmole) from New England Nuclear (Boston, MA). Atenolol, pronethalol hydrochloride, propranolol hydrochloride and practolol (I.C.I. Ltd., Macclesfield, U.K.) and acebutolol hydrochloride (May and Baker, Dagenham, U.K.) were generous gifts. Dimilume-30 and Soluene-350 were purchased from Packard Instrument Co. (Reading, U.K.). All other chemicals were obtained from B.D.H. (Poole, U.K.) in the purest form available.

All experiments were carried out on male Wistar albino rats (140–160 g body wt). Animals received daily oral administrations of the β-blocking drugs (5–200 mg/kg) for five days and were killed five or fifteen hours after last administration. Atenolol and practolol were administered in solution in 0.1 M citric acid/$Na_2HPO_4$ buffer, pH 6.5, and the remaining drugs were administered in water. The control group received the corresponding volume of drug vehicle.

Determination of the effects of β-blocking drugs on the synthesis of gastrointestinal mucus glycoprotein was carried by measuring the rates of incorporation of labelled sugar precursors into acid-precipitated glycoprotein, essentially as described by Lukie and Forstner [11]. Animals were killed by cervical dislocation and the stomach and intestine (15 cm from stomach) were immediately excised and cut open along the greater curvature and longitudinally, respectively, and washed with ice-cold 1.15% KCl solution. The mucosae were scraped onto a glass plate with a microscope slide, suspended in 10 ml of modified Krebs–Ringer bicarbonate solution and homogenised with a Potter–Elvehjem mechanically driven homogeniser. Aliquots of the homogenates (4.0 ml) were incubated with the radiolabelled sugar or amino acid for 2 hr at 37° in a shaking water bath. The medium was gassed with 95% $O_2$/5% $CO_2$ every 20 min. The reaction was terminated by addition of 5 ml of a 20% (w/v) solution of trichloroacetic acid containing 2% (w/v) phosphotungstic acid. The tubes were left to stand at 4° overnight and the white precipitate formed was deposited by centrifugation. The precipitate was twice washed with distilled water (5 ml) and twice with 5 ml of a mixture of chloroform–methanol (1:1, v/v) to remove non-pro-

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tein materials. The glycoprotein was dried in air and then transferred to glass scintillation vials and weighed. Distilled water (0.2 ml) and Soluene-350 (1.0 ml) were added, the vials were capped and placed in an oven at 50° until the protein was digested. After cooling, Dimilume-30 (10 ml) was added and the radioactivity was measured by scintillation spectrometry in an LKB-Wallac Ultrabeta 2002 scintillation counter. Blank preparations contained all the same components, but the radiolabelled sugars were added after the addition of the trichloroacetic acid–phosphotungstic acid mixture. The rate of incorporation of radiolabelled sugars into mucus glycoprotein was linear for incubation periods up to 3 hr. The precipitated mucus fraction has been shown by affinity chromatography on Sephadex to contain the label only in mucus glycoprotein [12].

## RESULTS

The effect of administration of the β-blocking drugs, acebutolol, atenolol, practolol, pronethalol and propranolol on the incorporation of *N*-acetylglucosamine into rat gastric mucosal glycoproteins is shown in Table 1. Acebutolol and atenolol had no significant effect, propranolol and pronethalol at very high dosage gave rise to an increased rate of the sugar incorporation but, in contrast, practolol at all doses led to a significant decrease in the incorporation of *N*-acetylglucosamine in gastric mucosal glycoproteins. Similarly, practolol inhibited the incorporation of fucose, *N*-acetylneuraminic acid and serine into gastric mucosal glycoproteins (Table 2). Propranolol and pronethalol had no effect on the incorporation of these labelled moieties, but acebutolol and atenolol increased the incorporation of serine and fucose, respectively.

Furthermore, although practolol administration showed a trend towards inhibition of the incorporation of *N*-acetylglucosamine, fucose and serine into intestinal mucosal glycoproteins (Table 3), none of these data were statistically significant.

## DISCUSSION

β-Adrenergic blocking agents may interfere with mucosal glycoprotein turnover through their effects on cyclic nucleotide levels [13–15]. Drugs which damage mucus may do so by interfering with its synthesis, its rate of secretion, and by altering its molecular complexity [16].

In the present study practolol has been seen to cause a marked inhibition of the incorporation of the precursor sugars and the amino acid, L-serine, into gastric mucosal glycoproteins. This indicates that practolol inhibits synthesis of the polypeptide core and its subsequent glycosylation. Practolol at doses below 50 mg/kg has no effect on the incorporation of sugar precursors into gastric mucosal glycoproteins (results not shown); the human dose for therapeutic purposes is 5 mg/kg. The other β-blocking drugs studied did not inhibit the incorporation of any of the glycoprotein precursors.

In the present study all β-blocking drugs were administered orally at the same dose, but the plasma concentrations attained are markedly different, as these drugs exhibit different absorption and metabolism characteristics [17]. Practolol is readily absorbed from the gastrointestinal tract and undergoes no significant first-pass effect, achieving approximately 100% bioavailability. In contrast the other β-blocking drugs employed in this study are poorly absorbed from the gastrointestinal tract or exhibit marked first-pass effects, and so achieve only 30–60% bioavailability [17]. However it is unlikely that the increased bioavailability of practolol is responsible for its inhibitory effect on glycoprotein synthesis as this was evident at a dose 50 mg/kg while with the other drugs no inhibition was seen even at 200 mg/kg.

It is not yet clear whether the adverse effects of practolol are due to the drug itself or to a toxic metabolite. Practolol is metabolized by liver microsomes of rat and hamster to metabolites which bind irreversibly to the microsomes [18], and practolol and its metabolites have been shown to bind to

Table 1. Effect of administration of β-blocking drugs on the incorporation of *N*-acetylglucosamine into rat gastric mucosal glycoproteins

| Dose (mg/kg) | Rate of [$^3$H]-*N*-acetylglucosamine incorporation (mole × $10^{-13}$/mg glycoprotein/hr) | | | | |
|---|---|---|---|---|---|
| | Acebutolol | Atenolol | Practolol | Pronethalol | Propranolol |
| 0 | 7.0 ± 0.7 | 9.8 ± 1.4 | 14.0 ± 1.2 | 10.1 ± 0.8 | 10.5 ± 0.9 |
| 50 | 6.9 ± 1.0 | 7.9 ± 1.5 | 9.6 ± 0.9* | 11.1 ± 0.9 | 11.2 ± 1.2 |
| 100 | 7.1 ± 0.6 | 7.1 ± 0.9 | 6.5 ± 0.7* | 15.7 ± 1.0* | 11.0 ± 1.6 |
| 200 | 7.7 ± 0.6 | 8.6 ± 0.9 | 8.0 ± 0.7* | 16.5 ± 1.8* | 13.5 ± 0.5* |

Results are presented as means ± S.E.M. of 3–6 determinations.

Animals were killed 15 hr after the last administration and mucosal homogenates were incubated with labelled *N*-acetylglucosamine (72.5 ng).

Differences in the control data may be attributed to biological differences in the experimental animals and to differences in specific radioactivity of the [$^3$H]-*N*-acetylglucosamine. The various doses of each drug were studied during the same experiment, carried out on the same day, but different drugs were studied on different days. In the experimants with acebutolol, atenolol, pronethalol and propranolol, [$^3$H]-*N*-acetylglucosamine of 3.0 Ci/mmole specific radioactivity was used, while in the experiment with practolol, another batch of [$^3$H]-*N*-acetylglucosamine of 4.7 Ci/mmole specific radioactivity was used.

* $P < 0.05$.

Table 2. Effect of administration of β-blocking drugs on the incorporation of glycoprotein precursors into gastric mucosal glycoproteins

| Drug | | Rate of incorporation (mole × $10^{-15}$/mg glycoprotein/hr) | | |
|---|---|---|---|---|
| | | [$^{14}$C]-*N*-Acetylneuraminic acid | [$^{3}$H]-D-Fucose | [$^{3}$H]-L-Serine |
| Acebutolol | Control | 4.5 ± 0.5 (4) | 6.9 ± 0.7 (6) | 16.8 ± 3.0 (4) |
| | Test | 5.0 ± 0.3 (4) | 6.5 ± 0.5 (6) | 26.9 ± 2.5* (5) |
| Propranolol | Control | 4.5 ± 0.5 (4) | 6.9 ± 0.7 (6) | 16.8 ± 3.0 (4) |
| | Test | 5.2 ± 0.6 (4) | 5.8 ± 0.5 (6) | 16.7 ± 1.5 (5) |
| Pronethalol | Control | 4.5 ± 0.5 (4) | 6.9 ± 0.7 (6) | 19.8 ± 2 (4) |
| | Test | 3.6 ± 0.5 (4) | 6.3 ± 0.4 (6) | 21.1 ± 1.5 (4) |
| Atenolol | Control | 11.1 ± 2.5 (4) | 4.5 ± 0.1 (5) | 46.6 ± 3.9 (5) |
| | Test | 6.4 ± 0.9 (4) | 5.9 ± 0.4* (4) | 37.1 ± 3.7 (5) |
| Practolol | Control | 11.1 ± 2.5 (4) | 9.4 ± 1.4 (5) | 46.6 ± 3.9 (5) |
| | Test | 4.7 ± 0.3* (4) | 5.7 ± 0.4* (5) | 29.8 ± 3.6* (5) |

Results are presented as means ± S.E.M. with the number of determinations given in parentheses.
Animals were dosed daily by gastric intubation with the drugs (150 mg/kg) for 5 days and killed 5 hr after last administration.
Mucosal homogenates were incubated with *N*-acetylneuraminic acid (323.4 ng), fucose (20.8 ng) and serine (60 ng).
* $P < 0.05$.

Table 3. Effect of practolol administration on the incorporation of glycoprotein precursors into intestinal mucosal glycoproteins

| Glycoprotein precursor | Rate of incorporation (mole × $10^{-15}$/mg glycoprotein/hr) Control | Test |
|---|---|---|
| [$^{3}$H]-*N*-Acetylglucosamine | 511 ± 86 | 452 ± 51 |
| [$^{3}$H]-D-Fucose | 6.3 ± 0.9 | 4.1 ± 0.3 |
| [$^{3}$H]-L-Serine | 4.9 ± 0.6 | 3.7 ± 0.5 |

Results are presented as means ± S.E.M. for four determinations.
Animals pretreatment and incubation conditions are as in Table 2.

tissues of the eye of hamster [19], although no ocular toxicity was reported following chronic administration to these animals. Practolol is slowly metabolised by aromatic ring hydroxylation [20], and this pathway of metabolism is known to be genetically controlled in man in respect of the drugs phenformin, perhexiline and debrisoquine [21, 22].

In conclusion, the present study demonstrates that administration of practolol at high dosage to animals inhibits gastric glycoprotein synthesis, which may impair the formation of mucus. Mucus is the natural lubricant and 'moisturiser' for many anatomical systems, and facilitates free intestinal movement within the peritoneal cavity, and contributes to protection of the eye [23]. Inhibition of mucus synthesis could therefore lead to intestinal adhesions and to 'dry eye' syndrome. Although practolol has been shown to inhibit mucus synthesis only in the gastric mucosa, because of the common pathways of mucus biosynthesis in various tissues, the effect of this β-blocking drug on the synthesis of peritoneal and ocular mucus synthesis merits investigation. However, it should be emphasized that only practolol had an inhibitory effect on gastric glycoprotein synthesis, and this occurred only at high dosage.



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