Effects of Propranolol on Atherogenesis in the Cholesterol-Fed Rabbit

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SUMMARY. These studies have examined the effects of dl-propranolol, d-propranolol, and metoprolol on aortic atherogenesis in the cholesterol-fed rabbit and have correlated the vascular effects of the drugs with their influence on blood pressure, plasma lipids and lipoproteins, arterial metabolism, and arterial permeability. dl-Propranolol and, to a lesser extent, d-propranolol, used in clinically relevant doses of 5 mg/kg body weight per day, inhibited the development of aortic atherosclerosis in association with significant reductions in aortic free and esterified cholesterol content. No significant effects of the drugs on blood pressure or on the total amounts or types of circulating lipoproteins were apparent. Accumulation of cholesterol in the liver and adrenal gland was not influenced by propranolol. Aortic acyl CoA:cholesterol acyltransferase and lysosomal enzyme activities were reduced by propranolol administration, but the inhibition may have been secondary to the lesser degrees of atherosclerosis and cholesterol accumulation present. In vitro inhibition of acyl CoA:cholesterol acyltransferase activity by either dl- or d-propranolol was also observed, but occurred only at propranolol concentrations of \(10^{-5}\) M or greater. Treatment with dl-propranolol had no significant effect on the rate of transport of labeled albumin across the isolated carotid artery of cholesterol-fed rabbits. Metoprolol administration (6.25 mg/kg body weight per day) had no significant influence on atherogenesis or arterial metabolism in this model. The results suggest that propranolol inhibits in part the development of atherosclerosis in the cholesterol-fed rabbit, and that the effect may be related to a direct action on the arterial wall. (Circ Res 56: 755-762, 1985)

SEVERAL drugs affecting sympathetic nervous function and used clinically to lower blood pressure may also inhibit the development of atherosclerosis in normotensive animals in response to cholesterol feeding. Propranolol, a \(\beta\)-adrenergic blocker, has been reported to retard atherogenesis in the cholesterol-fed rabbit (Whittington-Coleman et al., 1973). Similar effects have been observed in both rats and rabbits with reserpine (Smith and Rossi, 1962; Deming, 1966; Whittington-Coleman et al., 1968), a centrally acting adrenergic inhibitor, and in rabbits with guanethidine (Whittington-Coleman and Carrier, 1970), a peripheral sympathetic blocker. The mechanism for any anti-atherogenic action of these drugs in the cholesterol-fed rabbit has not been delineated, although several possibilities exist, including effects of the drugs on blood pressure, the renin-angiotensin system, the amount or properties of plasma lipoproteins, platelet function, or on the arterial wall itself through some direct action.

The current study has been performed to investigate the effects of \(\beta\)-adrenergic blockade on blood pressure, plasma lipids and lipoproteins, arterial metabolism, arterial permeability, and atherogenesis in the cholesterol-fed rabbit. Three drugs were selected as pharmacological probes to examine the problem: dl-propranolol, which is a potent antagonist of both \(\beta_1\) and \(\beta_2\)-receptor activity; d-propranolol, the optical isomer which has little \(\beta\)-blocking action except at high doses, but which has similar membrane-stabilizing activity as the racemic mixture; and metoprolol, a potent antagonist with membrane-stabilizing properties, but unlike propranolol, metoprolol has a weak inhibitory effect on the \(\beta_2\)-receptor, except at high doses.

**Methods**

**Animals**

Male New Zealand white rabbits (Charles River Breeding Laboratories) weighing between 1.5 and 2.0 kg were used in this study. Animals were housed individually with free access to food. The cholesterol-fed animals were fed a diet containing 2% cholesterol and 8% peanut oil for an 8-week period, while the control group received only commercial rabbit chow (Purina Co.).

We monitored systolic blood pressure every 2 weeks, using a tail cuff method developed in our laboratory (Kramsch et al., 1980). Measurements were made between 2 and 4 hours after drug administration. Unanesthetized rabbits were placed in a Styrofoam box with an opening for exposure of the tail. The tail was shaved, and the animal was acclimated to the box with head and eyes covered for 30 minutes before the measurements. A photoelectric sensing element was taped into place over the tail, and the systolic blood pressure was detected with an IITC MOD 59 preamplifier (Innovators in Instrumentation) and recorded by a Narco physiograph. The average
of five consecutive measurements was used for the determinations. Values varied less than 10 mm Hg between measurements.

Materials

\[1-[^{14}C]\text{oxy} \text{CoA (specific activity 43.4 Ci/mol)} \text{and tri-}[1-[^{14}C]\text{glycerol (specific activity 44 Ci/mol)} \text{were obtained from New England Nuclear Corp. N-[Nitrhophtenyl-N-acetyl-\(\beta\)-glucosaminide and \(p\)-nitrophenyl-\(\alpha\)-glucoside were obtained from Sigma Chemical Co. Egg yolk lecithin was a product of Lipid Products. dl-Propranolol and \(d\)-propranolol were kindly supplied by Ayerst Laboratories, and metoprolol was supplied by the Ciba-Geigy Company.}

Drug Treatment

Three separate sets of experiments were performed to evaluate the effects of \(\beta\)-blocker therapy on aortic atherosclerosis. In an initial study, only the influence of daily \(dl\)-propranolol in cholesterol-fed rabbit was assessed. In the second investigation, four groups of animals were studied: control, cholesterol-fed, cholesterol-fed with \(dl\)-propranolol, and cholesterol-fed with \(d\)-propranolol treatment, and the effects of therapy on arterial lipid metabolism as well as atherogenesis were examined. The third set of experiments examined the influence of metoprolol in treated and untreated cholesterol-fed rabbits. Propranolol or metoprolol was dissolved in sterile saline, and the \(pH\) was adjusted to 7.0. Each drug was administered once daily by intraperitoneal injection in a total volume of 1 ml beginning at the initiation of cholesterol feeding. The dose of \(dl\)- and \(d\)-propranolol was 5 mg daily, and of metoprolol, 6.25 mg daily. Paired cholesterol-fed rabbits were injected daily with the vehicle alone.

Plasma Lipids and Lipoproteins

Blood was obtained from the marginal ear vein at 1- to 2-week intervals. Previously described procedures were used for measuring plasma cholesterol (Rudel and Morris, 1973) and triglyceride (Fletcher, 1968). Plasma lipoproteins were analyzed after 4 weeks of cholesterol feeding in samples of blood drawn into EDTA after an overnight fast. Three milliliters of plasma were layered beneath a solution containing 0.195 M NaCl, 1 mM K2 EDTA, and 0.04% sodium azide and centrifuged at 49,000 rpm for 17 hours at 4°C in a 60 Ti rotor. The very low density lipoprotein (VLDL) fraction obtained was washed once and resuspended in 0.25 M sucrose, 0.01 M Tris, pH 7.4, and frozen at —20°C for subsequent lipid determinations. The remaining material was centrifuged at 10,000 g for 10 minutes, and the supernatant was centrifuged again at 100,000 g for 45 minutes. The resulting microsomal fraction was washed once and resuspended in 0.25 M sucrose, 0.01 M Tris, pH 7.4, and frozen at —80°C for subsequent chemical and enzymatic studies. The 100,000 g supernatant was used for the lysosomal enzyme assays. Tissue homogenates of the left adrenal gland and a segment of the right anterior lobe of the liver were also prepared in buffer, and aliquots were used for subsequent lipid determinations.

Enzyme Assays

Acyl CoA:cholesterol acyltransferase activity (ACAT) was determined in aortic microsomes by measuring the rate of incorporation of \(1-[^{14}C]\text{oleate (Brecher and Chan, 1980). In the standard assay, 15–20 \(\mu\)g of protein were incubated for 25 minutes at 25°C in 0.2 ml of solution containing 0.1 M potassium phosphate bufer (pH 7.5), 20 \(\mu\)M dithiothreitol, 0.3% of bovine serum albumin, and 5 \(\mu\)M \(1-[^{14}C]\text{oleoyl CoA}. The reaction was terminated by adding 4 ml chloroform:methanol (2:1, vol/vol) containing unlabeled cholesteryl oleate and oleic acid (10 mg/liter) as carriers, and a lipid extract was obtained (Folch et al., 1957). Cholesteryl-\(1-[^{14}C]\text{oleate was determined in the lipid extract by thin layer chromatography using hexane:dithyl etheracetic acid (90:10:1, vol/vol/vol) as developing solvent. Enzymatic activity was expressed as pmol of cholesteryl[\(1^{14}C\)]oleate formed per min per mg of microsomal protein. In selected experiments, where the in vitro effect of \(dl\)- and \(d\)-propranolol on ACAT activity was examined, the drugs were added directly to the assay system before the reaction was initiated.}

Neutral \(\alpha\)-D-glucosidase activity was determined in aortic microsomes as described (Brecher and Chan, 1980).

We assayed \(N\)-acetyl-\(\beta\)-glucosaminidase (NAGA) activity in the 100,000 g supernatant by measuring the release of \(p\)-nitrophenol resulting from the hydrolysis of \(p\)-nitrophenol-\(N\)-acetyl-\(\beta\)-glucosaminide, using incubation conditions reported previously (Brecher et al., 1978). Enzymatic activity was expressed as nmol \(p\)-nitrophenol released per min per mg of protein.

Acyl lipase activity was measured in the 100,000 g supernatant of aortic homogenate according to a slight modification of our previously published procedure (Brecher et al., 1977). Tricryglycerol-phospholipid vesicles (1.5 molar ratio) were prepared by sonication of a mixture of 60 mg of egg yolk lecithin, 12.5 mg triolein, and 8.0 \(\mu\)Ci \(1-[^{14}C]\text{glycerol and were employed as substrates. The assay of lipoprotein-\(p\)-nitrophenol CoA into cholesteryl oleoyl CoA into cholesteryl oleoyl CoA was weighed, minced, and homogenized in 10 volumes of sucrose-Tris buffer with a motor-driven, glass-glass homogenizer at 0–2°C. In the initial set of studies that examined the effects of \(dl\)-propranolol alone, the homogenates were assayed only for free and esterified cholesterol content. In the second and third group of experiments, a portion of the tissue homogenate was stored at —20°C for subsequent lipid determinations. The remaining material was centrifuged at 10,000 g for 10 minutes, and the supernatant was centrifuged again at 100,000 g for 45 minutes. The resulting microsomal fraction was washed once and resuspended in 0.25 M sucrose, 0.01 M Tris, pH 7.4, and frozen at —80°C for subsequent chemical and enzymatic studies. The 100,000 g supernatant was used for the lysosomal enzyme assays. Tissue homogenates of the left adrenal gland and a segment of the right anterior lobe of the liver were also prepared in buffer, and aliquots were used for subsequent lipid determinations.
pressed as nmol free fatty acid released per min, per mg protein.

**Chemical Analysis**

The protein content of aortic homogenates was determined by a micro-Kjeldahl procedure. Protein was determined on the microsomal fraction and 100,000 g supernatant as described (Lowry et al., 1951).

Lipids were extracted from tissue homogenates (Folch et al., 1957). Free and esterified cholesterol were separated by thin layer chromatography and their content analyzed using a Transidyne RFT-II recording densitometer under conditions where a linear relationship between peak area and the amount of cholesterol was obtained (between 0.3 and 8.0 μg material).

**Studies of Arterial Albumin Transport**

The influence of dl-propranolol on albumin transport across rabbit common carotid artery was also examined in cholesterol-fed rabbits using an experimental system recently developed in our laboratory (Chobanian et al., 1983). Studies were performed after 8 weeks of cholesterol feeding in the presence or absence of daily propranolol treatment (see above). Rabbits were anesthetized and the left common carotid artery exposed. Its branches were ligated and Silastic catheters were inserted at both ends to allow continuous perfusion in a closed system with solution consisting of Dulbecco’s modified Eagle’s medium to which was added 10% fetal calf serum, l-glutamine, and papaverine. [125I]Albumin (New England Nuclear Co.) was also added to the perfusion medium in concentration of 5 μCi/ml, and the solution was continuously recirculated through the carotid artery at approximately 50 mm Hg mean pressure. Blood samples were collected from a jugular venous catheter at intervals over a 4-hour period, and both the total [125I] radioactivity and that precipitated with trichloroacetic acid were determined. Radioactivity also was assayed in the intima-media preparation of the perfused carotid artery at the end of the study period. Four rabbits were used in each group.

Results were expressed as the mean ± se. The data in Tables 1–3 were analyzed statistically by one-way analysis of variance for each variable. When differences were significant, intergroup comparisons were done by Newman-Keuls test.

**Results**

**Effects of dl-Propranolol in Cholesterol-Fed Rabbits**

We performed pilot studies of groups of five cholesterol-fed rabbit treated for 8 weeks with either saline or dl-propranolol. Propranolol administration produced a marked decrease in total surface involvement of the aorta by visible atherosclerotic lesions, compared with the saline-treated controls, with disease in the propranolol group restricted primarily to the upper arch of the thoracic aorta and the regions surrounding the ostia of the intercostal arteries. The individual lesions in the treated and untreated groups appeared comparable when examined by light microscopy. Total aortic cholesterol content was reduced significantly by propranolol from 12.8 ± 0.6 to 4.9 ± 0.4 mg/g wet weight (P < 0.05).

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body wt (kg)</th>
<th>Blood pressure (mm Hg)</th>
<th>Heart/body wt (×10⁻³)</th>
<th>Aortic wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.7 ± 0.2</td>
<td>104 ± 7</td>
<td>1.9 ± 0.1</td>
<td>0.51 ± 0.08</td>
</tr>
<tr>
<td>Cholesterol-fed</td>
<td>2.9 ± 0.3</td>
<td>89 ± 6</td>
<td>1.9 ± 0.2</td>
<td>0.71 ± 0.10</td>
</tr>
<tr>
<td>Cholesterol-fed and dl-propranolol</td>
<td>2.5 ± 0.2</td>
<td>81 ± 6</td>
<td>1.8 ± 0.1</td>
<td>0.62 ± 0.08</td>
</tr>
<tr>
<td>Cholesterol-fed and d-propranolol</td>
<td>2.7 ± 0.3</td>
<td>88 ± 7</td>
<td>1.9 ± 0.1</td>
<td>0.67 ± 0.08</td>
</tr>
</tbody>
</table>

Results represent the means ± se for six animals.

Plasma cholesterol levels did not differ significantly between the two groups.

**Effects of dl- and d-Propranolol on Tissue Cholesterol and Aortic Enzyme Activity**

On the basis of the above findings, a more extensive study was performed involving four groups of rabbits: chow-fed controls, cholesterol-fed controls, cholesterol-fed rabbits treated daily with dl-propranolol, and cholesterol-fed animals treated with d-propranolol. Body weights did not differ significantly in the four groups, and no undesirable side effects due to propranolol treatment were observed. The systolic blood pressures were somewhat lower in all cholesterol-fed groups than in the control animals (Table 1). However, neither dl- nor d-propranolol in the dosage used produced a significant change in the blood pressure of the animals, compared to their cholesterol-fed controls. The ratios of heart weight to body weight were similar in all groups studied.

A rapid increase in the mean plasma cholesterol level occurred in response to the cholesterol diet, with values exceeding 1000 mg/dl by 3 weeks, and further rises to levels between 2000 and 2500 mg/dl by the end of the study. There were no significant differences in plasma cholesterol or triglyceride between the cholesterol-fed groups.

As in the pilot studies, the rabbits receiving dl-propranolol had visible aortic atherosclerosis restricted primarily to the upper arch of the thoracic aorta and to the ostia of the intercostal arteries. Somewhat greater involvement was apparent in the d-propranolol group, but the latter still was not as widespread as the extensive disease present in the untreated cholesterol-fed animals.

The aortic cholesterol data reflected the changes apparent by visual inspection. Aortic free and ester cholesterol contents were significantly increased above control levels in the three cholesterol-fed groups (Table 2). However, both dl- and d-propranolol treatment significantly decreased free and ester aortic cholesterol accumulation, although the d-iso-
mer appeared to have a lesser effect than the racemic mixture.

Marked increases in free and total esterified content of both adrenal gland and liver occurred in each of the cholesterol-fed groups (Table 2). Neither dl- nor d-propranolol had any significant influence on the accumulation of cholesterol in these organs, despite their effects on the aorta.

Enzymatic studies on aortic subcellular fractions from the four groups are summarized in Table 3. ACAT activity was increased 10-fold as a result of cholesterol feeding, but when dl-propranolol was also administered, the increase in aortic ACAT was attenuated. d-Propranolol treatment was not as effective in reducing aortic ACAT as the dl-isomer, and, in general, ACAT activity reflected aortic cholesterol content in all 3 cholesterol-fed groups. Neutral glucosidase activity, a microsomal marker enzyme, also was increased by cholesterol feeding, but the relative changes between the different groups were not clearly related to the incidence of atherosclerosis. Interestingly, if ACAT activity was expressed relative to glucosidase activity, the relative enhancement of ACAT activity was similar for the cholesterol-fed and cholesterol-fed plus d-propranolol groups. Lysosomal enzyme activity was assessed using NAGA and acid lipase as marker enzymes. Changes in the activities of both enzymes reflected the degree of atherosclerosis found for all groups treated. Acid lipase activity for the d-propranolol group was not measured.

Experiments were performed to determine if either dl- or d-propranolol influenced ACAT activity directly (Fig. 1). Addition of either compound to the in vitro assay system for ACAT over a wide range of concentrations resulted in a dose-dependent inhibition only at relatively high concentrations. A 50% inhibition was achieved at $10^{-3}$ M with both dl- and d-propranolol. No appreciable difference between the drugs was present at any concentration.

To determine whether propranolol treatment might alter the characteristics of the lipoproteins of cholesterol-fed rabbits, we performed a separate study to compare the d < 1.006 g/ml fraction from cholesterol-fed controls and cholesterol-fed rabbits given rf-propranolol. Plasma lipids were determined 4 weeks after initiating treatment. Plasma cholesterol and triglyceride were similar in both groups, with triglyceride levels less than 75 mg/100 ml in all rabbits from both groups. Over 75% of the total plasma cholesterol was localized in the d < 1.006 g/ml fraction. Figure 2A shows agarose gel electro-

| Table 2 | Effects of dl- or d-Propranolol on Serum and Tissue Cholesterol Content in Control and Cholesterol-Fed Rabbits |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Serum cholesterol (mg/dl) | Aorta | Liver (mg/g wet wt) | Adrenal |
|                | Free | Ester | Free | Ester | Free | Ester |
| Control        | 77 ± 6 | 1.8 ± 0.2 | ND | 2.2 ± 0.1 | 2.2 ± 0.2 | 6.6 ± 0.5 | 124 ± 10 |
| Cholesterol-fed | 2120 ± 165 | 6.6 ± 0.5 | 10.6 ± 1.2 | 8.7 ± 3.7 | 46.2 ± 3.7 | 9.7 ± 0.8 | 251 ± 18 |
| Cholesterol-fed and dl-propranolol | 2320 ± 211 | 2.8 ± 0.2† | 2.7 ± 0.3† | 9.9 ± 0.7 | 49.3 ± 4.1 | 11.1 ± 0.9 | 251 ± 21 |
| Cholesterol-fed and d-propranolol | 2701 ± 231 | 3.3 ± 0.3* | 6.7 ± 0.5† | 8.4 ± 0.6 | 54.0 ± 4.3 | 10.8 ± 0.8 | 272 ± 23 |

Results represent the means ± SE for six animals. ND = not detectable.

* Significantly (P < 0.05) different from cholesterol-fed group.
† Significantly (P < 0.01) different from cholesterol-fed group.

| Table 3 | Effect of Propranolol Treatment on Microsomal and Lysosomal Enzymes of Rabbit Aorta |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment       | ACAT (pmol/min per mg) | Neutral d-glucosidase | NAGA (nmol/min per mg) | Acid lipase |
| Control         | 4.0 ± 0.2 | 3.8 ± 0.2 | 1.6 ± 0.2 | 55 ± 2.0 |
| Cholesterol-fed | 43.1 ± 2.5 | 9.8 ± 0.8 | 8.2 ± 0.9 | 148 ± 5.1 |
| Cholesterol-fed and dl-propranolol | 14.2 ± 3.1† | 6.0 ± 0.5† | 3.7 ± 0.4† | 78 ± 3.1† |
| Cholesterol-fed and d-propranolol | 29.0 ± 3.4† | 7.2 ± 0.4* | 7.5 ± 0.3 |

Results represent the means ± SE for six animals. ND = not detectable.
* Significantly (P < 0.05) different from cholesterol-fed group.
† Significantly (P < 0.01) different from cholesterol-fed group.
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PROPRANOLOL (M)

**FIGURE 1.** In vitro effects of dl-propranolol (closed circles) and d-propranolol (open circles) on incorporation of [1-14C]oleyl CoA into cholesteryl-[14C]oleate. The drug was added directly to the assay system prior to initiating the reaction.

phoretograms of representative plasma samples from both groups, indicating that the major lipoproteins had a β migration typical of β-VLDL. The apoprotein profiles of the d < 1.006 g/ml fractions, determined using polyacrylamide gel electrophoresis (Fig. 2B), showed that in both groups, apo-B and apo-E were clearly the major apoproteins present. Collectively, the data in Figure 3 indicate that β-VLDL, the major lipoprotein of cholesterol-fed rabbits, also was the predominant cholesterol-containing particle in the dl-propranolol-treated group.

The studies of 125I-albumin transport across the rabbit common carotid artery are summarized in Table 4. Comparisons were made between cholesterol-fed animals that received either metoprolol (6.25 mg/kg per day), intraperitoneally, or the saline vehicle. Heart rate was significantly lower in the metoprolol-treated animals (175 ± 14 beats/min), compared with either control (230 ± 7) or cholesterol-fed untreated rabbits (217 ± 8). Blood pressure appeared unaffected by metoprolol (87.3 ± 6.3 mm Hg in controls, 90.3 ± 8.4 in the cholesterol-fed untreated group, and 90.6 ± 7.1 with metoprolol therapy). Similarly, no significant differences in either aortic free or ester cholesterol, ACAT, or NAGA activity were present (Fig. 3).

**TABLE 4**

<table>
<thead>
<tr>
<th>Plasma 125I radioactivity (dpm/ml per hr)</th>
<th>dl-propranolol (5 mg/kg per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol-fed</td>
<td>3850 ± 1225</td>
</tr>
<tr>
<td>Cholesterol-fed + propranolol</td>
<td>5830 ± 2850</td>
</tr>
</tbody>
</table>

Values represent the means ± se for four separate studies. *After 4-hour perfusion of carotid artery with 125I-labeled albumin.

than control, chow-fed rabbits that we had previously studied (Chobanian et al., 1983).

**Effects of Metoprolol Therapy**

A separate set of experiments was performed to study the influence of metoprolol on atherogenesis and arterial metabolism. Comparisons were made between cholesterol-fed animals that received either metoprolol (6.25 mg/kg per day), intraperitoneally, or the saline vehicle. Heart rate was significantly lower in the metoprolol-treated animals (175 ± 14 beats/min), compared with either control (230 ± 7) or cholesterol-fed untreated rabbits (217 ± 8). Blood pressure appeared unaffected by metoprolol (87.3 ± 6.3 mm Hg in controls, 90.3 ± 8.4 in the cholesterol-fed untreated group, and 90.6 ± 7.1 with metoprolol therapy). Similarly, no significant differences in either aortic free or ester cholesterol, ACAT, or NAGA activity were present (Fig. 3).

**Discussion**

These studies demonstrate that propranolol inhibits the development of aortic atherosclerosis in cholesterol-fed rabbits and confirms the prior findings of Whittington-Coleman et al. (1973). Interestingly, dl-propranolol which has minimal β-receptor-block-
ing effects, except at large doses, also appeared to have an anti-atherosclerotic action, although to a lesser extent than the racemic mixture. Metoprolol, on the other hand, appeared to have no influence on atherogenesis in this model, indicating that the propranolol response is not necessarily shared by all β-adrenergic inhibitors. Several possible explanations for these results need to be considered.

A blood pressure-lowering action or some other hemodynamic-related effect of propranolol could conceivably play a role in inhibiting atherosclerosis. Other antihypertensive medications, including guanethidine (Whittington-Coleman and Carrier, 1970), reserpine (Smith and Rossi, 1962; Deming, 1966), and the calcium channel blocker, nifedipine (Henry and Bentley, 1981), have been reported to have similar protective action in the cholesterol-fed rabbit. However, in the dosages that we used, which were based on the average amount of drug typically administered to human patients, no significant lowering of systolic blood pressure could be demonstrated beyond that induced by cholesterol feeding alone. In addition, metoprolol, which should be expected to have an influence on blood pressure or cardiac contractility similar to that of propranolol, offered no protection to the animals. Propranolol has been reported to produce a reduction in blood velocity in rhesus monkeys (Spence et al., 1977). Such an effect could potentially reduce shear stress on the arterial endothelium, but its relationship to atherogenesis is unknown.

Sympathetic nervous function may influence the response of the arterial wall to potentially injurious stimuli such as hypertension or hyperlipoproteinemia. Sympathetic de-innervation of rabbit ear arteries has been shown to reduce both the growth of the artery and DNA synthesis in medial smooth muscle cells (Bevan and Tsuru, 1981). Furthermore, sympathetic de-innervation of cerebral arteries in the spontaneously hypertensive rat can minimize the expected increases in wall thickness and elevate markedly the risk of developing strokes (Sadoshima et al., 1981). Although it is appealing to consider that the effect of propranolol is somehow related to its influence on vascular sympathetic activity, the findings with d-propranolol and metoprolol would not support such an explanation.

We also examined the effects of propranolol on plasma lipid and lipoprotein levels and on the nature of the circulating lipoproteins. Although no influence on total plasma cholesterol and triglyceride levels in the rabbit had previously been noted, it was possible that some critical change in the lipoproteins themselves might be induced by the drug. In fact, we recently observed in another model, the alloxan-diabetic cholesterol-fed rabbit, that protection against atherosclerosis occurred despite even higher levels of total plasma cholesterol and triglyceride. Such protection appeared attributable to the formation of an unusual particle with properties of a chylomicron remnant which became the major circulating lipoprotein rather than β-VLDL (Brecher et al., 1983). In the current study, chemical and electrophoretic analysis of the d <1.006 g/ml lipoprotein fraction from d/-propranolol-treated rabbits indicated the presence of the β-VLDL particle characteristic of that typically found in cholesterol-fed rabbits. Recent work has shown that β-VLDL interacts with macrophages leading to cholesteryl ester accumulation in these cells (Goldstein et al., 1980; Mahley et al., 1980), and it has been suggested that β-VLDL particles also interact with cells within the vessel wall leading to foam cell formation (Pitas et al., 1983). The determinants thought to be necessary for the atherogenic properties of β-VLDL are a core of cholesteryl ester and the presence of apoprotein E on the surface. The presence of β-VLDL as the major cholesterol-carrying lipoprotein in propranolol-treated animals suggests that the effects of the drug on lesion development are not due to alterations in the properties of this potentially atherogenic lipoprotein.
We cannot exclude the possibility that some other important effect of propranolol on arterial lipid metabolism may have occurred. Prior in vitro studies have demonstrated that propranolol can inhibit several hydrolytic enzymes including rat liver phospholipase A2 and phospholipase C (Matsuzawa and Hoestler, 1980), as well as platelet phospholipase A2 (Vanderhoek and Feinstein, 1979). In addition, decreased phosphatidylcholine synthesis from \(^{32}\)P-labeled phosphate has been reported with propranolol (Pappu and Hansen, 1982). The significance of these findings, however, is uncertain. In our own studies, the changes observed in aortic ACAT activity or in lysosomal enzymes reflected the degree of atherosclerosis and are probably not related to direct effects of propranolol.

Both \(l\)- and \(d\)-propranolol have effects on cell membranes which appear unrelated to their \(\beta\)-blocking action. Such membrane-stabilizing action has been reported for several tissues, including cardiac muscle (Langslet, 1970). Of particular interest with respect to atherogenesis is the action of propranolol on platelet aggregation. A marked reduction in sensitivity to platelet aggregation induced by adenosine diphosphate (ADP), epinephrine, collagen, and thrombin has been observed with both isomers in vitro (Weksler et al., 1977) while an inhibition of platelet aggregation and thromboxane synthesis in patients receiving long-term propranolol also has been noted (Campbell et al., 1981). However, metoprolol also appears to share membrane-stabilizing and platelet actions (Campbell et al., 1981), and it is unlikely that differences between the drugs can be explained on this basis.

Studies with erythrocyte membranes suggest that propranolol interacts with membrane protein (Singer, 1977) or lipid (Godin et al., 1976) and could conceivably influence a variety of properties of cells, including their permeability. We recently demonstrated, using a model of the isolated rabbit common carotid artery perfused in vivo, that cholesterol feeding of even brief duration markedly increased albumin flux across the vessel (Chobanian et al., 1983). We therefore considered whether propranolol might affect such transport. However, no significant effect of the drug on albumin transport could be demonstrated in this model after 8 weeks of cholesterol feeding. It is possible that shorter periods of study might be required to demonstrate such an effect. As noted, propranolol does not prevent entirely the development of atherosclerosis, and even mild changes in the arterial intima might cause profound effects on permeability.

Inhibition of plasma renin activity and circulating levels of angiotensin II also could theoretically be beneficial to the vasculature. High levels of angiotensin II, when associated with hypertension, may be vasculotoxic, particularly to small arteries and arterioles (Giese, 1973). Hypertensive patients with low plasma renin activity were reported to be relatively protected from developing myocardial infarcts or strokes (Brunner et al., 1972), but more recent clinical evidence has not supported this hypothesis (Kaplan, 1975). In addition, recent studies in cholesterol-fed rabbits do not support a role of renin on atherogenesis in this model (Overturf et al., 1981a, 1981b). In addition, metoprolol is a potent inhibitor of plasma renin activity and still afforded no protection against cholesterol feeding.

Other studies have demonstrated that the cholesterol-fed rabbit can be a useful model for studying the relationship between hyperlipoproteinemia and atherosclerosis, but the possibility exists that metabolic differences are present between rabbits and humans which might confound the interpretation of the data as related to their clinical relevance. For example, lipid deposition in reticuloendothelial tissue and a hemolytic anemia can occur frequently in the cholesterol-fed rabbit, but rarely if ever in hyperlipoproteinemic man. How such differences might influence our findings is unknown.

The recent observation that ablation of the sinoatrial node in male cynomolgus monkeys reduces the extent of coronary atherosclerosis after cholesterol feeding (Beere et al., 1984) raises the possibility that the effects of propranolol that we observed may somehow be related to its chronotropic action. We do not consider this to be likely in view of the beneficial effects of \(d\)-propranolol, which did not slow heart rate, and the lack of anti-atherosclerotic effect of metoprolol, which, however, reduced heart rate to an extent induced by \(dl\)-propranolol.

The clinical implications of our findings cannot be assessed by the current study, although major interest has developed recently in the long-term effects of antihypertensive drugs on coronary artery atherosclerosis and its complications. Some large clinical trials have suggested that mortality related to ischemic heart disease may not be reduced by drug therapy (VA Cooperative Study Group, 1970; Management Committee ATMM, 1980) and that high-dose diuretic treatment may possibly increase coronary-related deaths (MRFIT Research Group, 1982). Also, diuretic drugs can have an adverse effect on plasma lipids and lipoproteins by increasing plasma low density lipoproteins (LDL) and VLDL, while some \(\beta\)-blockers may lower plasma high density lipoproteins (HDL) (Weidmann et al., 1983). On the other hand, propranolol has been shown to reduce the rate of reinfarction in patients surviving a myocardial infarct (BHAT Study Group, 1981). The current investigation provides no information regarding these issues. To our knowledge, the only published study examining the effects of propranolol on atherogenesis in primates was by Pick and associates using stumptail Macaque monkeys (Pick and Glick, 1977). Their findings were equivocal in that their initial results suggesting an anti-atherogenic action of propranolol could not be confirmed by their subsequent investigation.
In conclusion, these studies have demonstrated that propranolol has a potent atherosclerosis-inhibiting action in the cholesterol-fed rabbit which does not appear to be related to its antihypertensive or β-blocking properties. It has been suggested that this drug has a direct effect on the arterial wall, although the exact mechanism remains to be defined.

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References

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