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THE EFFECTS OF PROPRANOLOL ON THE ARTERIAL RESPONSES TO HYPERTENSION AND HYPERCHOLESTEROLEMIA

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 [1]. 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 [2]. Furthermore, previous studies have suggested that several drugs affecting sympathetic nervous function may inhibit atherogenesis in normotensive animals in response to cholesterol feeding [3—5].

In view of these findings, we have performed a series of studies to determine whether inhibition of sympathetic or adrenergic function with the beta adrenergic inhibitor, propranolol, might have a direct influence on the arterial wall in experimental models of hypertension and hyperlipoproteinemia. The studies in the hypertensive animals have utilized the uninephrectomized rat treated with deoxycorticosterone and salt and have examined the effects of propranolol on the DNA content of arterial smooth muscle cells. The studies in hyperlipoproteinemic animals have investigated the influence of propranolol on the development of atherosclerosis in rabbits fed a diet containing 2% cholesterol and 8% peanut oil.

Materials and Methods. Studies in Hypertensive Rats. Male Wistar rats (175—200 gm) were uninephrectomized and one week later were divided into three groups: 1) Normotensive controls, 2) Animals treated with deoxycorticosterone pivalate (15 mg/kg given by subcutaneous injection biweekly) and 1% saline as drinking water (DOC-salt), and 3) Animals treated with deoxycorticosterone and saline as noted above but in addition given propranolol 500 mg/liter (80.6 ± 2.5 mg/kg/day) in the drinking water. Systolic blood pressure was measured using the tail cuff method as previously described [6] at the initiation of the study and at two-week intervals thereafter.

Aortic smooth muscle cells (SMC) were isolated by enzymatic dispersion techniques as described [7, 8].

Graphic display of the distribution of the diploid and tetraploid nuclei was obtained and the percent tetraploid nuclei determined [8].

Studies in Hyperlipoproteinemic Rabbits. Male New Zealand white rabbits weighing between 1.5 and 2.0 kg were used in this study. 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.). Systolic blood pressure was monitored every 2 weeks, using a tail cuff method developed in our laboratory [9]. 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. Propranolol was dissolved in sterile saline, and 5 mg were administered once daily by intraperitoneal injection throughout the 8-week period.

Acyl CoA: cholesterol acyltransferase (ACAT) was determined in aortic microsomes as described [10]. Acid lipase and N-acetyl-B glucosaminidase (NAGA) activities were measured in the 100.000 g supernatant as previously reported from our laboratory [11, 12].

Protein content of the homogenates was determined by a microkjeldahl procedure and of the microsomal and 100.000 g supernatants by the Lowry method [13]. Lipids were extracted from tissue homogenates [14], free and ester cholesterol were separated by thin-layer chromatography, and their content was analyzed using a Transidyne RFT—II recording densitometer.

Results

Studies in DOC-salt Hypertensive Rats. There was a significant rise in systolic blood pressure in the DOC-salt hypertensive group when compared to control rats (Table 1), but there were no statistically significant differences in blood pressure between the animals treated with DOC-salt alone versus the DOC-salt hypertensive group treated with propranolol.

Heart and aortic weights (expressed as a percentage of body weight) increased significantly above control levels in both the DOC-salt hypertensive group and the DOC-salt hypertensives treated with propranolol. However, there was no significant difference in heart or aortic weight between these two groups. This shows that propranolol had no effect in preventing the increase in aortic or cardiac mass associated with the hypertension.

Figure 1 shows a typical computer-generated plot of fluorescence of aortic SMC nuclei. Table 1 summarizes the results of the ploidy determinations of the three groups. As expected from our previous work [8], there was an increase in the percentage of tetraploid nuclei in the DOC-salt hypertensive animals. However, propranolol prevented the development of polyploidy despite the hypertension such that there was no statistically significant difference between the DOC-salt hypertensive group treated with propranolol and the normotensive controls.

Effects of Propranolol in Cholesterol-Fed Rabbits. Most of the data have been reported in a previous publication by us [9]. Body weights did not differ significantly in the four groups of rabbits, 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, but neither dl—nor d-propranolol produced a significant change in blood pressure in the cholesterol-fed controls. The ratios of heart weight to body weight were similar in all groups studied.

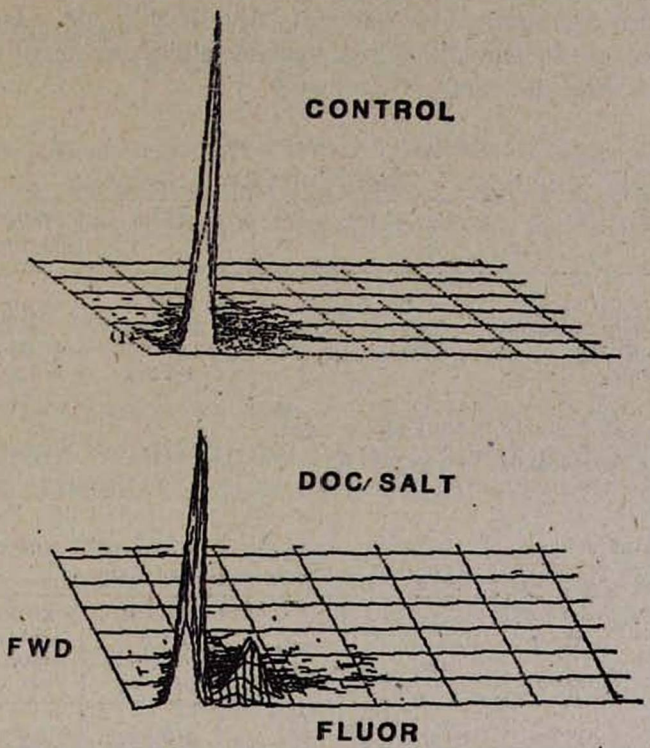

Figure 1. Representative plots of fluorescence and forward light scatter for arterial smooth muscle cell suspensions stained with propidium iodide. Cells were prepared from a control rat (A) and a hypertensive rat treated with DOC-salt for 4 weeks (B).

A rapid increase in the mean plasma cholesterol level occurred in response to the high 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. Lipoprotein and apolipoprotein profiles of the d<1.006 fraction of plasma assayed by agarase and polyacrylamide gel electrophoresis did not show differences between any of the cholesterol-fed groups.

Administration of dl—propranolol produced a marked decrease in total surface involvement of the aorta by visible atherosclerotic lesions as compared with the cholesterol-fed controls. The disease in the propranolol group was restricted primarily to the aortic arch and to regions surrounding the ostia of the intercostal arteries. Somewhat greater involvement was apparent in the d-propranolol group but was not as wides-

pread 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 were significantly increased above control levels in all cholesterol-fed groups (Table 2). However, both dl—and d-propranolol treatment significantly decreased free and ester cholesterol accumulation in the aorta although the d-isomer had a lesser effect than the racemic mixture.

Table 1

Effects of Propranolol on Nuclear Polyploidy in the DOC-salt Hypertensive Rat

	Control	DOC—salt	DOC—salt and Propranolol
Systolic BP, 4 wk	122,0±3,0	167,0±8,0	160,0±14,0
Heart weight, % BW	0,24±0,006	0,39±0,005	0,34±0,10
Aortic weight, % BW	0,024±0,001	0,039±0,002	0,036±0,002
Tetraploid, cells %	7,8±0,3	17,0±0,2	8,9±0,9

BP=Blood Pressure; BW=Body Weight

Results represent the means±S. E.

Table 2

Effects of Propranolol on Serum and Aortic Cholesterol and on Aortic Enzyme Activities in Cholesterol-Fed Rabbits

	Serum Cholesterol, (mg/dl)	Aortic Cholesterol (mg/g wet wt) Free	Ester	Aortic ACAT, pmol/min per mg	Enzyme NAGA, nmol/min per mg	Activities Acid Lipase
Control	77±6	1,8±0,2	ND	4,0±0,2	1,6±0,2	55±2,0
Cholesterol-fed	2120±165	6,6±0,5	10,6±1,2	43,1±2,5	8,2±0,9	148±5,1
Cholesterol-fed and dl-propranolol	2320±211	2,8±0,2*	2,7±0,3*	14,2±3,1*	3,7±0,4*	78±3,1*
Cholesterol-fed and d-propranolol	2701±231	3,3±0,3**	6,7±0,5*	29,0±3,4*	7,5±0,3	—

Results represent the means±S. E. for six animals. ND=not detectable.
* Significantly ($P<0.01$) different from cholesterol-fed group.
** Significantly ($P<0,05$) different from cholesterol-fed group.

ACAT activity was increased 10-fold by cholesterol feeding but such increase was attenuated dy propranolol, particularly the racemic preparation (Table 2). In general, ACAT activity reflected aortic cholesterol content in all three cholesterol-fed groups. Activity of lysosomal enzymes using NAGA and acid lipase as marker enzymes also appeared to reflect the degree of atherosclerosis present for all groups tested.

Conclusions. These studies have demonstrated that propranolol inhibits the development of nuclear polyploidy in aortic smooth muscle cells of DOC-salt hypertensive rats and that the effect is unrelated to its blood-pressure-lowering action. They also have indicated that pro-

pranolol has a potent suppressive effect on atherogenesis in the cholesterol-fed rabbit. Independent of any influence on blood pressure or plasma lipoproteins. The findings suggest a direct effect of the drug on the arterial wall, possibly by inhibiting beta receptor activity of vascular SMC.

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ՀԻՊԵՐՏՈՆԻԱՅԻ ԵՎ ՀԻՊԵՐԽՈԼԵՍՏԵՐՈԼԵՄԻԱՅԻ ԺԱՄԱՆԱԿ ՋԱՐԿԵՐԱԿՆԵՐԻ ՌԵԱԿՑԻԱՆ ՊՐՈՊՐԱՆՈԼՈԼԻ ԱԶԴԵՑՈՒԹՅԱՆ ՆԿԱՏՄԱՄԲ

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Հետազոտությունները ցույց են տվել, որ պրոպրանոլոլը ուղղակիորեն ազդում է զարկերակների պատի վրա անոթային բջիջների բետա-ռեցեպտորների արգելակման միջոցով։

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ВЛИЯНИЕ ПРОПРАНОЛОЛА НА РЕАКЦИЮ АРТЕРИЙ ПРИ ГИПЕРТОНИИ И ГИПЕРХОЛЕСТЕРИНЕМИИ

Проведенные исследования показали, что пропранолол имеет прямое воздействие на стенки артерий, ингибируя активность бета-рецепторов в сосудистых клетках.

ЛИТЕРАТУРА

1. *Bevan R. D., Tsuru H.* Circ. Res., 49, 478—485, 1981. 2. *Sadoshima S., Busija D., Boody M., Heistad D.* Hypertension 3, 1—124—1—127, 1981. 3. *Whittington-Caleman P. J., Carrier O. Jr.* Atherosclerosis 12, 15—24, 1970. 4. *Whittington-Coleman P. J., Carrier O. Jr., Douglas B. H.* Atherosclerosis, 18, 337—345, 1973. 5. *Smith T. H. G., Rossi G. V.* J. Pharmacol. Exp. Ther. 135, 367—373, 1962. 6. *Haudenschild C. C., Prescott M. F., Chobanian A. V.* Hypertension, 2, 33—44, 1980. 7. *Ives H. E., Schultz G. S., Galardy R. E., Jamieson J. D.* J. Exp. Med. 148, 1400—1413, 1978. 8. *Lichtenstein A. H., Brecher P. I., Chobanian A. V.* Hypertension, 8, 11—50—11—54, 1986. 9. *Chobanian A. V., Menzolan J. O., Shipman J., Heath K., Haudenschild C. C.* Circ. Res., 53, 805—814, 1983. 10. *Brecher P., Chan C. T.* Biochim. Biophys. Acta 617, 458—471, 1980. 11. *Brecher P., Pyun H. Y., Chobanian A. V.* J. Lipid Res. 18, 154—163, 1977. 12. *Brecher P., Chan C. T., Franzblau C., Faris B., Chobanian A. V.* Circ. Res. 43, 561—569, 1978. 13. *Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J.* J. Biol. Chem., 193, 265—275, 1951. 14. *Folch J., Lees M., Sloane-Stanley G. M.* J. Biol. Chem., 226, 497—509, 1957.