Effect of Prednisone on the Noradrenaline-Induced Cell Proliferation in the Arterial Wall

Recent morphological observations on the large elastic arteries of man and animals have firmly established that numerous injurious factors may stimulate DNA synthesis and division of the smooth muscle cells of the tunica media[1–13]. Previous autoradiorgraphic and colchicine studies from this laboratory have shown that in the adrenalinetreated rabbits the cells composing the media of the aorta and the main pulmonary artery are actively synthetizing desoxyribonucleic acid, and further go on to divide[14,15]. In the present study we have investigated whether such proliferation occurs also after the administration of another naturally occurring catecholamine, i.e. noradrenaline. Further, it seemed interesting to investigate whether the arterial proliferation of the catecholamine-treated rabbits may be modified by glucocorticoids, i.e. prednisone.

Adult male rabbits, weighing about 2 kg, were used. The animals were fed a semisynthetic well-balanced diet and housed in individual cages at room temperature. The study included 2 groups; one noradrenaline group of 10 animals received i.v. L-noradrenaline bitartrate, 100 µg/kg body wt. daily, for 30 days. Another group of 10 animals was treated with L-noradrenaline as above and concomitantly received prednisone i.m., 1 mg/kg body wt daily, for 30 days. At the end of the experimental period, 1 h before the sacrifice, all the rabbits were injected i.v. with 1 mCi/kg of body wt of ^{3}H-thymidine. At autopsy the entire aorta and the main pulmonary artery were removed, opened ventrally and inspected for gross alterations. Selected segments of the vessels were fixed in 4% neutral formaldehyde and processed partly for light microscopy and partly for autoradiography using the usual techniques. On autoradiographs the mean number of labelled nuclei per mm^2 of surface area of the undamaged media was calculated. In some untreated control animals, it was found that normally the large elastic arteries of the rabbit incorporate little or no ^{3}H-thymidine.

On macroscopic inspection, only the aorta showed gross changes. The changes affected primarily the arch, the ascending and the thoracic aorta and, as a rule, appeared as round or elongated white plaques 2 to 5 mm in dia-

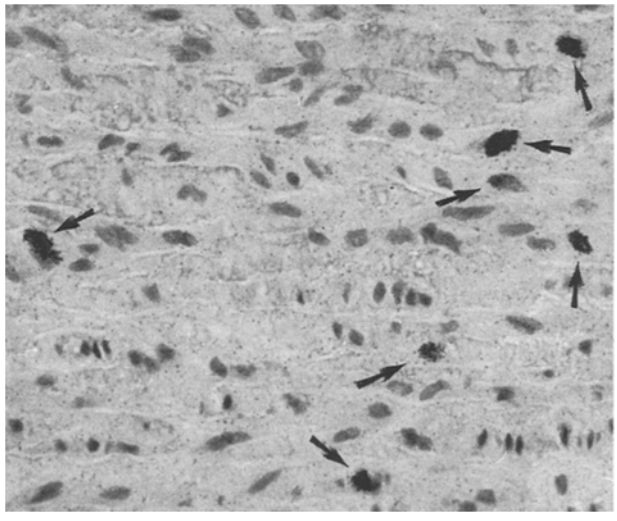

Fig. 1. ^{3}H-thymidine labelled nuclei (arrows) in the aortic media of a noradrenaline-prednisone treated rabbit. × 250.

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Table I. Lesions of the media in noradrenaline and in noradrenaline-prednisone treated animals, histologically assessed

Noradrenaline		Noradrenaline and prednisone	
Pulmonary	Aorta	Pulmonary	Aorta
+	++	+	++++
++	++	+	++
–	++	++	+++
+	+	++	++++
++	++	++	++
+	+	++	++
++	++++	++	+++
++	++	++	++
++	++	++	++++
++	++++	++	+++

+ scattered intercellular accumulations of PAS-positive material; ++ small necrotic foci; +++ granular deposits of calcium; ++++ large calcified plaques.

Table II. Number of labelled nuclei per mm^2 of medial surface in noradrenaline and noradrenaline-prednisone treated animals

	Noradrenaline		Noradrenaline and prednisone	
	Pulmonary	Aorta	Pulmonary	Aorta
	n.d.[a]	1.10	3.36	3.51
	0.27	0.42	n.d.	3.22
	n.d.	0.39	4.80	3.05
	0.38	0.82	2.90	2.60
	5.23	2.42	3.10	2.47
	2.87	5.46	3.90	2.92
	2.33	1.36	5.99	12.10
	0.12	0.26	5.30	n.d.
	1.70	2.15	3.28	4.44
	0.90	1.35	n.d.	14.62
Means	1.72 ± 0.282	1.57 ± 0.223	4.09 ± 1.581	5.43 ± 2.379

[a] Not determined.

meter, solitary or grouped. In some cases the entire aorta was transformed into a stiff tortuous, dilated tube. In the noradrenaline group 3 out of 10 aortae showed clear-cut aneurysmatic dilatations of the proximal segments of the vessel; in the noradrenaline-prednisone group the lesions were evidently more advanced, 6 out 10 animals showing extensive aneurisms frequently reaching the abdominal aorta.

In both groups of animals the main histological changes in the pulmonary artery were represented by accumulations of PAS-stainable intercellular material in the subendothelial spaces and in the innermost layers of the tunica media; in some cases small necrotic foci were seen. Calcifications were never observed. Instead, in the aorta, besides mucoidosis and widespread necroses of the medial smooth muscle, deposition of calcium was a common finding in the necrotic areas of the tunica media as well as more diffusely in the amorphous ground substance in between the elastic membranes. Stretching and splitting of the elastic lamellae at the site of the calcifications and a slight increase in the amount of collagen in the immediate surroundings of the calcified lesions were observed. In agreement with the gross changes, the microscopic changes were more severe and diffuse in the noradrenaline-prednisone group than in the noradrenaline group (Table 1).

Table II shows the results of ^{3}H thymidine studies. From this Table it appears that long-standing noradrenaline treatment leads to the appearance of ^{3}H labelled nuclei in the tunica media of both the aorta and the main pulmonary artery. Further, it appears that ^{3}H thymidine incorporation is significantly increased by the simultaneous administration of prednisone. In both groups of animals the labelling was particularly evident in the surroundings of the necroses and calcifications. In the single cases a rough correlation was found to exist between the severity of the medial lesions and the rate of ^{3}H-thymidine uptake. From the results reported above it appears that i.v. noradrenaline treatment may induce in the rabbit vascular lesions which are quite similar to those elicited by adrenaline; actually the main changes observed have been necrosis of smooth muscle cells and focal or diffuse deposition of calcium salts[16-18]. Concomitantly, as shown by the autoradiographic study, the uninvolved smooth muscle of the tunica media proliferates in an attempt to repair and restore functional and structural integrity of the arterial wall. In agreement with others, it was found that the noradrenaline-induced arteriopathy is greatly facilitated by the accompanying administration of prednisone[19,20]; besides, under steroid treatment the nuclear DNA synthesis in the medial coat of the arteries was found to be significantly increased. This latter finding was rather unexpected since it is widely taken for granted that glucocorticoids are potent inhibitors of cell reactions and proliferation. Consequently, the enhancing effect of prednisone on the noradrenaline-induced arteriopathy cannot be related to an inhibitory effect on the reparative proliferation of the well-differentiated smooth muscle cells of the media, but more likely to an effect of the glucocorticoid on the extracellular components of the arterial wall[21].

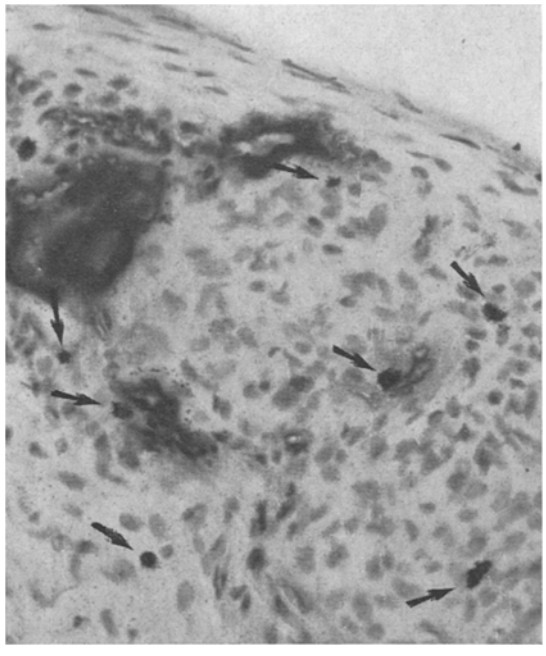

Fig. 2. Aorta of a noradrenaline-prednisone treated rabbit showing numerous labelled nuclei (arrows) in the surroundings of a medial calcified plaque. × 250.

Riassunto. Si è studiata la morfologia e la proliferazione cellulare che si instaura nella media dell'aorta e dell'arteria polmonare di conigli sottoposti a trattamento con noradrenalina e con noradrenalina-prednisone facendo uso del metodo autoradiografico dopo somministrazione di ^{3}H timidina. Le lesioni e la proliferazione della muscolatura della media che si attuano in seguito a somministrazione di noradrenalina vengono sensibilmente aggravate dal trattamento contemporaneo con prednisone.

C. CAVALLERO, U. DI TONDO, M.N. PERICOLI and P. SARTI

Institute of Pathological Anatomy II, University of Rome, Viale Regina Elena 324, I-00161 Roma (Italy), 10 November 1972.

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Lymphocyte-Stimulating Activity of Scarlet Fever Toxin

There is some indirect evidence suggesting that the biological activity of scarlet fever toxin is partially dependent on previous sensitization of the organism[1,2]. If this is so, cell hypersensitivity should be demonstrable by the test of blastic transformation of lymphocytes in vitro, which moreover allows distinction – on the basis of the quantitative parameters of the response – between the reaction of the sensitized lymphocyte to a specific antigen and the reaction to nonspecific mitogens.

Scarlet fever (erythrogenic) toxin (ET) was produced in vitro by the group A β-haemolytic streptococcus strain NY Dochez 5 and was prepared and purified essentially