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SOME PATHOGENETIC FACTORS IN EXPERIMENTAL "INDOMETHACINE" HYPERTENSION

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The concentrations of cyclic nucleotides (AMP and GMP) in the blood plasma, urine, and tissues, and morphological changes in the blood vessel walls of the kidneys were studied in rats with arterial hypertension induced by chronic inhibition of prostaglandin synthesis. Considerable thickening of the walls of the interlobular and arcuate arteries and marked constriction of the lumen, mainly on account of hypertrophy and swelling of smooth-muscle cells, were observed. Meanwhile the cyclic GMP concentration was increased, the cyclic AMP level lowered, and the cyclic AMP/cyclic GMP ratio reduced in the biological fluids. It is suggested that changes in the metabolism of the cyclic nucleotides are connected with organic and functional changes in the peripheral vascular system which lie at the basis of the increased general vascular resistance associated with arterial hypertension. KEY WORDS: indomethacine; prostaglandin; cyclic nucleotides; smooth-muscle cells; arterial hypertension.

In a paper published previously two forms of arterial hypertension (AH) were described in rats following administration of indomethacine, an inhibitor of prostaglandin (PG) synthesis, after unilateral nephrectomy or salt loading [1]. The antihypertensive effect of PG may be connected with their local action at the level of the peripheral vascular system, changes in which are characteristic of all forms of AH. It has been shown that PG counteracts the vasoconstrictor effect of the pressor hormones and blocks the secretion of catecholamines by nerve endings [6, 7].

The ultimate physiological action of PG in the tissue and, in particular, in the smooth-muscle cells of the blood vessels is mediated through a system of cyclic nucleotides [3]. It has been shown that cyclic AMP has a marked relaxant effect, and cyclic GMP a vasoconstrictor action [3, 5, 9]. It is also interesting to note that cyclic nucleotides have opposite effects on protein synthesis and on cell proliferation in certain tissues [2, 4].

The object of this investigation was to compare the cyclic AMP and GMP levels in biological fluids and tissues with the morphological state of the peripheral vessels in rats against the background of chronic inhibition of PG synthesis.

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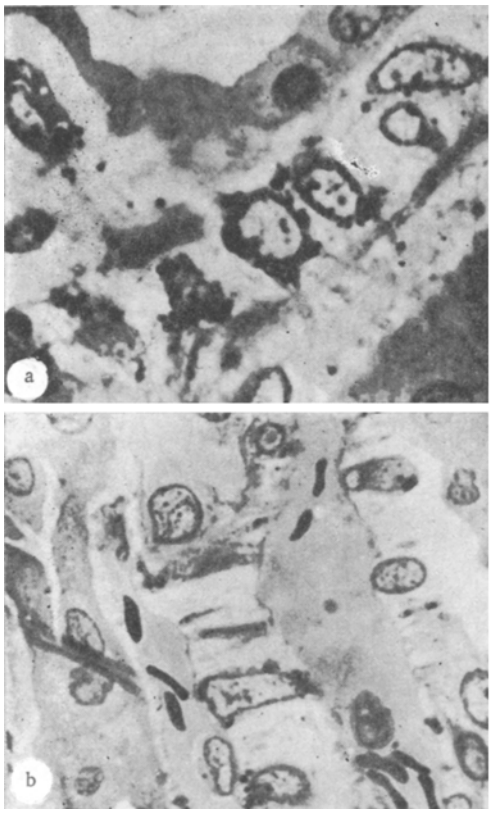

Fig. 1. Inner medullary layer of the kidneys and interstitial cells in rats of group 1 (a) compared with control (b). Stained with azure-2 and methylene blue, 1025×.

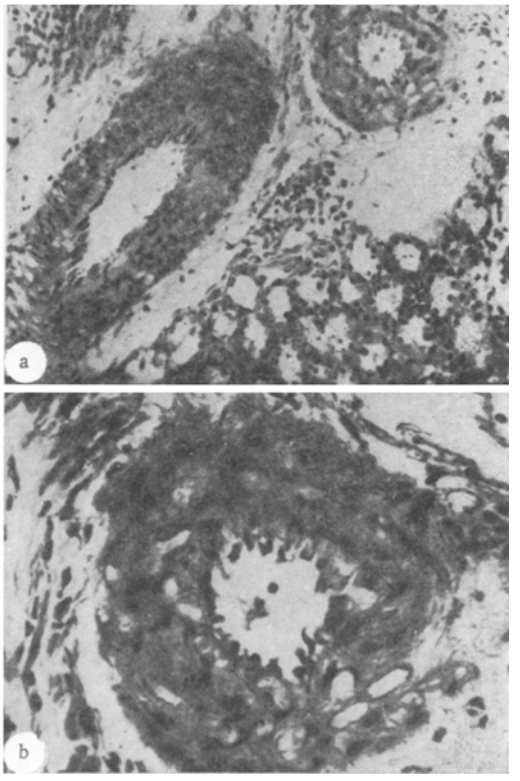

Fig. 2. Histological analysis of renal arteries in rats of group 1: a) interlobular and arcuate artery of kidney (420×); b) interlobular artery of kidney. Hematoxylin-eosin, 520×.

EXPERIMENTAL METHOD

Experiments were carried out on 56 male Wistar rats divided into the following groups: 1) 20 rats receiving 1% NaCl combined with daily administration of indomethacine in a dose of 2 mg/kg, 2) 15 rats receiving indomethacine after unilateral nephrectomy, 3) 10 control rats receiving saline to drink, 4) 6 nephrectomized rats, and 5) 6 intact rats. The duration of the experiment was six weeks. After decapitation of the animals' pieces of kidney tissue were fixed in 10% neutral formalin and embedded in paraffin wax. Histological sections were stained with hematoxylin-eosin, orcein, and by the PAS method. The tissues of the renal cortex and medulla were prefixed in 5% glutaraldehyde and then fixed in 1% OsO_4, and then embedded in a mixture of

TABLE 1. Concentrations of Cyclic Nucleotides in Blood of Control and Experimental Rats (M ± m)

Group of animals	Cyclic AMP, pmoles/ml	Cyclic, GMP, pmoles/ml	Cyclic AMP/ cyclic GMP
1 Indomethacine 2 mg/kg + 1% NaCl to drink	23,2±2,4 $P_{1-5}<0,05$	11,6±1,52 $P_{1-5}<0,001$	2,45±0,54 $P_{1-5}<0,001$
2 Unilateral nephrectomy + indomethacine	7,2±1,4 $P_{2-4}<0,001$	5,2±1,3	1,5±0,5 $P_{2-4}<0,05$
3 1% NaCl to drink	43,0±2,36 $P_{3-5}<0,05$	6,5±1,14	8,2±0,67
4 Unilateral nephrectomy	22,5±3,6	6,0±0,8	3,7±0,7 $P_{3-5}<0,01$
5 Intact animals	33,1±2,06	4,2±0,83	9,5±1,4

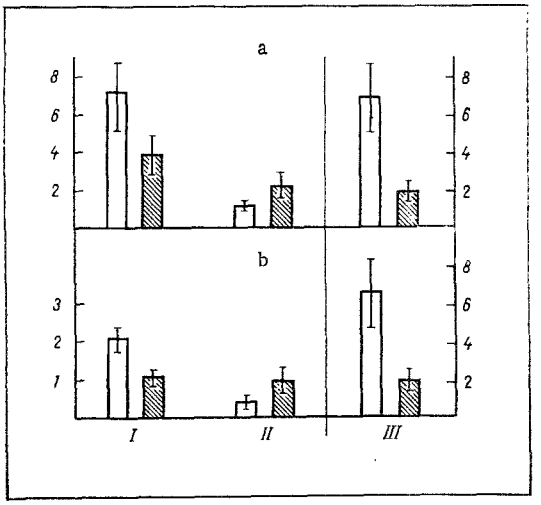

Fig. 3. Concentration of cyclic nucleotides in tissues of intact and unilaterally nephrectomized rats. a) Kidney; b) aorta. I) Cyclic AMP; II) cyclic GMP; III) cyclic AMP/cyclic GMP. Unshaded columns represent intact animals, shaded columns six weeks after unilateral nephrectomy. Ordinate: left, concentration of cyclic nucleotides (in pmoles/mg protein); right, cyclic AMP/cyclic GMP ratio.

Epon and Araldite. Semithin sections were stained with azure-2 and methylene blue to study the renal medulla. The concentrations of cyclic nucleotides in the blood plasma, urine, and tissues were determined by a radioimmunological method, using kits from the Radiochemical Centre, Amersham, England.

EXPERIMENTAL RESULTS

Toward the end of the experiment the arterial pressure was raised on the average to twice its normal height in 10 of the 20 rats of group 1 and in 9 of the 15 rats of group 2. The kidneys of the animals of group 1 were enlarged compared with those of the rats of groups 5 and 3. The ratio between the weight of the kidney and the body weight of the animal, for instance, was 3.7 in the rats of groups 1 and 2, compared with 3.2 in the rats of group 3 and 3.0 in those of group 5 ($P < 0.01$). Histological examination of the kidneys of these animals revealed marked hypertrophy of the epithelium of the distal tubules, especially of the ascending limb of Henle's loop and the collecting tubules, as well as marked hypertrophy and hyperplasia of the interstitial cells of the medulla (Fig. 1). In some glomeruli focal thickening of the basement membrane and sclerosis and thickening of the mesangium were observed. Considerable changes were found in the intrarenal vascular system. The arterial walls were considerably thickened with marked narrowing of their lumen (Fig. 2a). Hypertrophy of the smooth-muscle cells of the tunica media, with hyperchromasia and polymorphism of the nuclei were found. The cytoplasm of the smooth-muscle cells were swollen and vacuolated (Fig. 2b). In the kidneys of the animals of group 2, besides marked hypertrophy of all parts of the nephron, considerable sclerotic changes in the mesangium were found in some glomeruli, with periglomerular sclerosis around them. Changes in the intrarenal vessels were similar in character to those in the kidneys of the rats of group 1. The changes described above in the blood vessels of the kidneys were observed only in animals with AH; in experimental rats whose arterial pressure remained normal, no changes were observed in the vascular system.

Investigation of cyclic nucleotides in the blood of the animals gave the following result (Table 1). In rats receiving only a saline drink (group 3) the cyclic AMP concentration was raised and the cyclic GMP level remained relatively stable. The cyclic AMP/cyclic GMP ratio remained substantially unchanged. Administration of indomethacine to the animals receiving salt (group 1) caused a marked increase in the cyclic GMP concentration, and this was reflected in a sharp decrease in the cyclic AMP/cyclic GMP ratio. The concentration of cyclic AMP in the blood of the animals with unilateral nephrectomy (group 4) was significantly reduced, whereas that of cyclic GMP was increased a little compared with intact rats. Administration of indomethacine to the nephrectomized animals (group 2) led to an even greater decrease in the blood level of cyclic AMP and a considerable decrease in the cyclic AMP/cyclic GMP ratio.

Changes of a similar character were observed in the levels of the cyclic nucleotides in the urine of the experimental animals also. In intact rats the cyclic AMP/cyclic GMP ratio was 5.12 ± 0.61; in rats receiving NaCl to drink it was 4.67 ± 0.72, whereas in hypertensive animals receiving salt and indomethacine it fell to 2.46 ± 0.44 ($P < 0.05$). The concentration of cyclic nucleotides in the biological fluids is known to reflect changes in their metabolism in the tissues and, in particular, in the renal vascular system. Investigation of concentrations of these nucleotides in the tissues of the kidney and aorta of the control and nephrectomized rats shows a marked decrease in the cyclic AMP concentration and an increase in cyclic GMP in animals with one kidney compared with intact rats (Fig. 3).

The models of AH in rats described above are thus characterized by marked proliferation and hypertrophy of the smooth-muscle cells of the vessel walls and also by a considerable increase in the cyclic GMP concentration in the body and an accompanying decrease in cyclic AMP. Cyclic GMP is known to stimulate cell proliferation, whereas cyclic AMP inhibits it [4, 5]. This state of affairs is also confirmed by the increase in the cyclic GMP concentration discovered in the hypertrophied solitary kidney in rats. Similar results have been obtained by other workers studying cyclic nucleotide metabolism in the residual kidney after nephrectomy [8]. An absolute and relative (compared with cyclic AMP) increase in the cyclic GMP concentration in the body may lead to depression of the sodium pump and thereby bring about hypernatriemia and hyperhydration of the tissues, as well as an increase in the sensitivity of the vessel walls to the action of vasoconstrictors [3]. Disturbances of metabolism found in the vascular wall changed the ratio of wall to lumen and, if generalized, was an increase in the peripheral resistance.

It can be concluded from analysis of the results that functional and morphological changes arising in the peripheral vascular system of animals with the forms of arterial hypertension studied are directly connected with a disturbance of cyclic nucleotide metabolism.

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