It will be clear from Table 1 that GA and KGA had virtually no effect on the magnitude of the Bohr effect ($\Delta \lg p_{50}/\Delta pH$) and that only PA caused a statistically significant decrease in this value. Reduction of the Bohr effect by about 20% is evidence that addition of PA partly involves the groups responsible for the Bohr effect (α-terminal amino groups, β = 146 histidine and β = 122 histine). PA also had the strongest effect on interaction with Cl^- ions and KGA the weakest. On the whole, by analyzing the functional properties of these Hb derivatives it can be concluded that modification by KGA leads to no significant changes in the functional characteristics of native Hb, whereas addition of PA and GA causes changes in these characteristics, which bring them close to values obtained for normal whole blood.

These experiments thus show that irreversible addition of keto-acids to Hb affects the total charge on the protein, and this is reflected in its isoelectric properties, and also changes the character of interaction of Hb with allosteric effectors (O_2, Cl^-, H^+). This fact may be used to obtain modified Hb derivatives with affinity for oxygen similar to that of normal whole blood.

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PROSTAGLANDIN SYNTHETASE ACTIVITY IN LAYERS OF THE KIDNEY OF YOUNG RATS RECEIVING POLYENE ANTIBIOTICS

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UDC 612.46.015.1:577.152.6].014.46:615.33:577.182.66

KEY WORDS: prostaglandin synthetase, polyene antibiotics

Among the extensive group of drugs which cause severe injury to the structure and functions of the kidneys, the polyene antibiotics (PA) deserve special attention [1, 9, 11]. The mechanisms of the nephrotoxicity of PA remain virtually unexplained, although data recently obtained in the writers' laboratory suggest that the damaging effects of PA may be based on a disturbance of prostaglandin (PG) biosynthesis in different parts of the kidneys [6].

To test this hypothesis we studied the effect of PA on PG-synthetase (PGS) activity in the cortex, medulla, and papillary layer of the kidneys in young rats.

EXPERIMENTAL METHOD

Male rats weighing 180-200 g were used. The animals were divided into four groups. Rats of the control group received an intravenous injection of physiological saline in 5%

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(Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 3, pp. 302-304, March, 1986. Original article submitted January 29, 1985.

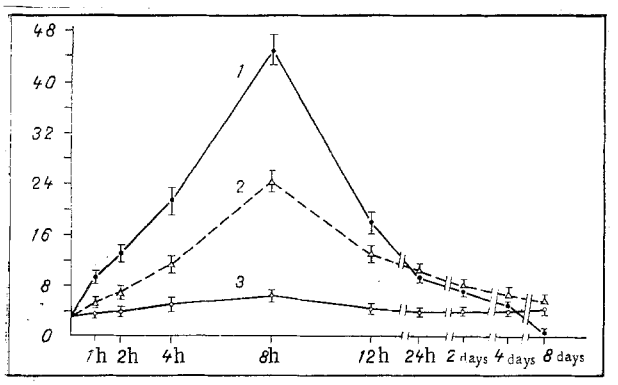

Fig. 1. Time course of changes in PGS activity in renal cortex under the influence of amphotericin B (1), levorin (2), and nystatin (3). Abscissa, time of taking samples for analysis; ordinate, conversion of ^{14}C-arachidonic acid into ^{14}C-PG (in %).

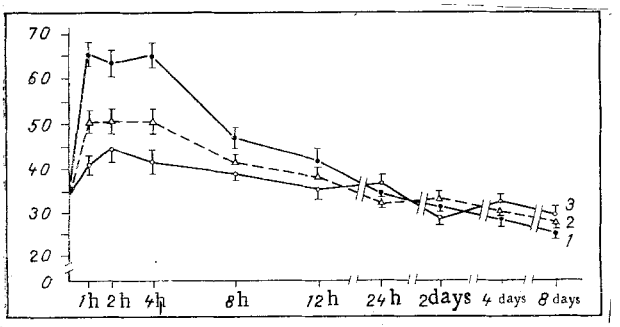

Fig. 2. PGS activity in renal medulla under the influence of PA. Legend as to Fig. 1.

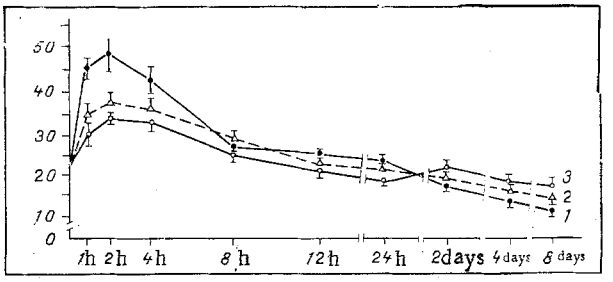

Fig. 3. Effect of PA on PGS activity in papillary substance of the kidney. Legend as to Fig. 1.

glucose into the caudal vein, rats of the remaining three groups received amphotericin B (250 U/kg), levorin (550 U/kg), and nystatin (700 U/kg) respectively. The animals were decapitated at different times after the beginning of antibiotic therapy (1, 2, 4, 8, 12, and 24 h, 2, 4, and 8 days). The kidneys were quickly removed from the abdomen and fixed in liquid nitrogen. They were then kept at –40°C. Before analysis the kidneys were thawed, cut into slices 2-3 mm thick perpendicularly to the long axis of the organ, and the cortical, medullary, and papillary zones were quickly separated. The tissue was ground in liquid nitrogen. Weighed samples of frozen powder (each containing 100-200 mg) were then prepared and placed in incubation medium in a volume of 1 ml, containing 0.1M Tris-HCl (pH 8.0) and 0.5 μCi/ml of the sodium salt of ^{14}C-arachidonic acid (55.5 mCi/mmole, England). Samples were incubated during shaking in a "Rotor-Mixer-Evaporator" (Buchler, USA) for 20 min at 37°C. Under these conditions the reaction is linear for 30 min. The reaction was stopped by addition of 200 μl of 5.6% formic acid and the samples were extracted twice with 5 volumes of ethyl acetate. After centrifugation the pooled organic phases (extract of PG) were evaporated. The residue was treated with 0.2 ml of 2.5% methanol solution in chloroform. To obtain the total fraction of ^{14}C-PG the method of column chromatography on silicic acid (Bio-Rad Laboratory, USA, 100-200 mesh), in the modification in [4], was used.

Part of the extract was introduced into a scintillation flask to determine total radioactivity, and the remaining total reaction extract was applied to a microcolumn, through which 2 ml of chloroform was passed to remove neutral lipids of methanol in chloroform eluted fractions containing all ^{14}C-PG directly into scintillation flasks. The eluates were evaporated to dryness and, after addition of toluene scintillator, their radioactivity was determined on a Mark III scintillation counter (USA). The difference between the total radioactivity before application of the sample and after removal from the column represented the total conversion of ^{14}C-arachidonic acid into ^{14}C-PG. The resulting radiactivity was expressed in percentage conversion of ^{14}C-arachidonic acid per gram net weight of tissue. The results were subjected to statistical analysis [2].

EXPERIMENTAL RESULTS

The time course of changes in PGS activity in the renal cortex under the influence of amphotericin B, levorin, and nystatin is illustrated in Fig. 1. All antibiotics caused on the whole consistent changes in PG activity, but the degree of these changes differed. For instance, amphotericin B increased PGS activity in the cortex 12-fold after 8 h, whereas levorin increased it 6.5-fold and nystatin 1.7-fold. At the end of observation (after 8 days) PGS activity under the influence of amphotericin B was 3.4 times less than in the control, whereas under the influence of levorin it was 1.8 times greater than the control. Nystatin increased PGS activity by only 1.3 times.

The data in Fig. 2 reflect changes in PGS activity in the renal medulla under the influence of PA. The action of amphotericin B and levorin reached a maximum 1 h after injection, and nystatin 2 h after injection. The fall in PGS activity in the renal medulla took place more smoothly than in the cortex. At the end of observation (8th day) amphotericin reduced activity of the enzyme by 1.4 times, levorin by 1.3 times, and nystatin by 1.2 times.

Finally, the time course of changes in PGS activity in the papillary substance of the kidney after injection of PA is illustrated in Fig. 3. PGS activity reached a maximum in this case after 2 h. During this period amphotericin B increased activity of the enzyme by 2.3 times, levorin by 1.7 times, and nystatin by 1.59 times compared with the control.

Analysis of the data thus shows considerable differences in the action of amphotericin B, levorin, and nystatin on the PG-synthesizing system in different layers of the kidney in young rats.

Only isolated data on the effect of antibiotics on PG synthesis may be found in the literature. For instance, several investigators [10] found activation of PG synthesis under the influence of adriamycin, whereas others, on the contrary, observed its inhibition through the action of cycloheximide [8]. From this point of view it was very convenient to link the nephrotoxic effect of the PA with their damaging action on PG synthesis. Choice of this hypothesis is supported by investigation of those workers [7] who have shown that the mechanism of action of many drugs is realized through the PG-system of the organs and tissues. It has recently been postulated that the nephrotoxic action of PA is based on their ability to bind with a cholesterol component of cell membranes [1, 3].

Amphotericin B, which has two double bonds in its structure, combines more "rigidly" with cholesterol then the other two antibiotics. In the opinion of some investigators this leads to a shift in the cholesterol/phospholipid ratio and sharply modifies the activity of the majority of membrane-bound enzymes, thereby influencing the permeability of lysosomal membranes and many biosynthetic processes in the cells [3, 5]. Effects of this kind can be prevented by certain PG, which have a marked stabilizing action on lysosomal membranes and reduce the release of proteolytic enzymes into the surrounding medium.

Investigation of PG activity still does not give a complete picture of the action of antibiotics on synthesis of individual types of PG and other active metabolites of arachidonic acid. However, the differences which we found in their effect on the PG-synthesizing system of the cell, through which many hormones and drugs realize their action, are evidence of distinct changes in the spectrum of PG synthesized in the kidneys. This may play an essential role in the harmful action of PA on function of the renal nephron.

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STATE OF COLLAGEN METABOLISM DURING IMMOBILIZATION AND ELECTROPUNCTURE

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UDC 616.153.962.9-02:613.863-02:612.766.2+615.844.4]-092.9

KEY WORDS: parameters of collagen metabolism; stress; immobilization; electropuncture

Analysis of the parameters of collagen metabolism is widely used to assess the state of the connective tissue in various diseases. However, little attention has been paid to the study of changes in the metabolism of the corresponding biopolymers which may be induced by the accompanying stress.

It was accordingly decided to study parameters of collagen metabolism in the course of repeated immobilizations and electropunctures, which were used as strong and weak stressors respectively.

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 150-200 g; 16 intact rats served as the control. The experimental animals were immobilized for 8 h daily in constricting cages for up to 5 days (series I, 30 rats) or subjected to electropuncture for 20 min (4-5 V, 50 Hz) in the zone of the stomach, by the method of Zakhar'in and Gede, for up to 8 days (series II, 40 rats). Some animals were exposed to electropuncture of the skin, accompanied after the 4th day of the experiment by simultaneous immobilization, as described above (series III, 30 rats). Electropuncture was applied 1 h before immobiliation. The apparatus used in the experiments was built at Ustinov Mechanical Institute.

The animals were killed under ether anesthesia at different stages of the experiments. Proteolytic activity (PA) was determined in the blood plasma [8]. Parameters of collagen metabolism, namely free, peptide-bound, and protein-bound hydroxyproline (FH, PEBH, and PRBH respectively) — in the blood plasma were studied with the aid of chloramine B and T and p-dimethylaminobenzaldehyde [2, 3, 6], as described later. Blood plasma in a volume of 2 ml was mixed with 1 ml of 6% TCA and 1 ml of 57% $HClO_4$. The mixture was centrifuged for 5-6 min at 1000 g. Exactly half of the supernatant was neutralized with 6 N NaOH until a pale purple color was obtained with phenolphthalein (tube 1). The other half was hydrolyzed in a boiling waterbath for 40 min, cooled, and neutralized (tube 2). The residue was treated with 1 ml each of distilled water, 6% TCA, and 57% $HClO_4$. The resulting mixture was hy-

Department of Biochemistry, Ustinov Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 3, pp. 304-306, March, 1986. Original article submitted April 25, 1985.

 0007-4888/86/0003-0306$12.50