PATHOLOGICAL AND MORPHOMETRIC EVALUATION OF TAURINE THERAPY IN A MODEL OF AORTIC INSUFFICIENCY IN RABBITS

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The traditional treatment of congestive heart failure (CHF) is based on the triad of cardiac glycosides, diuretics, and peripheral vasodilators. A course of treatment with these drugs is quite often complicated by side effects, with the appearance of forms of CHF refractory to this kind of treatment, and this is the reason why new therapeutic preparations are being sought. One such preparation is taurine (T), a natural 2-amino-ethanol-sulfonic acid present in mammalian organs and tissues and easily synthesized. To judge from all the facts, T is a universal neurotransmitter stimulating hematopoiesis, and modulating ion and cyclic nucleotide exchange, in particular in the myocardium and blood vessel walls [1, 2, 8, 4, 5], and has proved to be a highly effective cardioprotector in the treatment of experimental and clinical forms of CHF [6, 7]. It has been shown that T possesses cardiotonic, anticonvulsant, antiarrhythmic, and hypotensive activity, and putentiates the action of cardiac glycosides, and that these effects are mediated, in particular, by Ca^{2+} cations, in phospholipid complexes present in the membranes of cardiomyocytes (C) and leiomyocytes (L) of the vessel wall [12, 9, 10, 11].

However, there are no data in the literature on the pathological and morphometric evaluation of the action of T either experimentally or clinically. The aim of this investigation was to make good this deficiency by the use of modern stereomorphometric methods [3].

EXPERIMENTAL METHOD

The test objects were 65 male Chinchilla rabbits weighing 2.0-2.5 kg: 20 control and 45 experimental animals. Of the 20 control animals, 10 were not subjected to any manipulations, but the other 10 were anesthetized and a plastic catheter introduced into the common carotid artery to measure the hemodynamic parameters. Euthanasia was carried out on the animals after intravenous barbiturate (50 mg/kg) or urethane (600 mg/kg) anesthesia, by massive exsanguination, and considering the uniform nature of the changes characteristic of posthemorrhagic anemia, this enabled interpretation of the autopsy findings to be standardized. In 45 experimental rabbits, under general anesthesia, after measurement of the hemodynamic parameters, the operation of transcarotid aortic valvulotomy was performed, by means of a special needle, with soldered extension, 12 cm long and 3 mm in diameter, with perforation of one cusp of the aortic valve. The permanent regurgitation of blood from the aorta into the left ventricle thus produced led to volume overloading and hyperfunction of the left ventricle, with an increase of 75% in the pulse pressure compared with its initial value. Distinct ECG evidence of hypertrophy of the left and, later, of the right ventricle was recorded 1 month after the operation, and the animals developed CHF. Of the 45 rabbits four died from postoperative complications, and the other 41 developed marked aortic insufficiency with CHF. Of this number 14 received no treatment (survival control of the model) and the other 27 were treated. Daily for 1.5-2 months they received 0.5 g of potassium orotate (nine rabbits), 10 mg/kg of T (nine), and 100 mg/kg of T (nine) perorally with their food. At the end of the experiment, 3 months after the operation, surviving animals were subjected to euthanasia by exsanguination under general anesthesia, and im-

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mediately thereafter a complete autopsy was carried out on each animal in accordance with a special scheme, with recording of the pathomorphometric findings for each individual rabbit, a morbid anatomical diagnosis, and conclusion. Linear measurements were made with a millimeter tape. The cadavers of the animals were weighed on electronic scales (DINA, USSR). The organs were weighed with an accuracy equivalent to the fourth mark, on electronic scales (Sartorius, West Germany). Pieces of tissue were excised from all systems and organs in accordance with the GLP system or they were studied whole, after fixation in 10% neutral formalin. After fixation and washing in water, the material was defatted, tanned, and consolidated in alcohols of increasing concentration, and xylol, and embedded in paraffin wax with the aid of automatic microprocessor or vacuum histological systems (ATV, USSR; Sakura, Japan). Serial tissue sections (2-5 μ) were cut on rotary microtomes (Reichert, Austria; AO-820, USA). The sections were stained with hematoxylin and eosin, with fuchselin-picrofuchsine, by Masson's trichrome method, with cresyl- and crystal-violet, aldehyde-fuchsine, and toluidine blue (pH 3.2-7.4-10.2); the PAS reaction (with amylase and diastase) also was carried out. The stained sections were mounted in Canada balsam. The preparations were examined in a universal microscope (NU-2, East Germany), and microfilmed (Press-Photo, East Germany) on KN-2 film (USSR). Stereomicrometric measurements were made with ocular-(K-15x) and objective-(63x) micrometers (Jena, East Germany), after which, to standardize the investigation, they were made in serial sections with the aid of a modified Fibrecheck microimage analyzer (Vickers, Great Britain). The histoarchitectonics of the heart were studied in histotopograms of the organ passing through the septum and both ventricles, the so-called middle ring. Besides determination of all the splanchnomorphometric parameters of the mass of the organs, 45 cardiostereomorphometric tests also were studied: 19 linear, eight relating to the mass of each part, five indices derived from them, and 13 surface-volume micrometric indices. In this way an objective cardiomorphometric evaluation of all the observations in the control and in the model and the results of its treatment, could be undertaken. Particular attention was directed to the ratio of the mass of the heart and the body weight (CI — cardiac index), the mass of the left ventricle and of the heart (ILV — index of the left ventricle), the mass of the right ventricle and heart (IRV — index of the right ventricle), and the ratio between the masses of the ventricles (VI — ventricular index), and among the stereomicrometric parameters — to the diameter, surface area, relative volume, nucleosarcoplasmic index, and number of cells in 1 mm^2 area (K and F — fibroblasts). All splanchno- and cardiomorphometric parameters were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The total of 65 animals was divided into six groups. Group 1) 20 rabbits undergoing neither operation nor treatment, control for establishment of splanchno-cardiomorphometric norms. Besides posthemorrhagic anemia, which was common to all the animals, and which was excluded from analysis in the model and the results of its treatment, no changes whatever were found in their internal organs. Group 2) four of the 45 postoperative rabbits, which died from postoperative complications (the VSD syndrome, thromboembolism of the pulmonary and cerebral arteries, massive blood loss, suppurative mediastinitis with empyema). Because of their different pathology and the absence of T therapy, these were excluded from analysis. Group 3) 14 rabbits developing aortic insufficiency with CHF after aortic valvulotomy. To study the survival and mortality rates of the model, the animals of this group were not treated. Seven rabbits survived 3 months after the operation (natural survival rate 50%), whereas seven died 1.5-2 months after the operation from marked CHF with exhaustion, effusions into the body cavities, cardiosclerosis, and stage III-IV of circulatory failure (c.f.; mortality 50%). In the seven rabbits which survived, but in a clinically and biochemically enfeebled state, pathomorphometric investigation revealed the presence of CHF with stage II of c.f., although without signs of cachexia. The venous congestion, thrombohemorrhagic disturbances in the organs, anasarca, and effusions in these animals were initial and of slight degree, as also were the cirrhosis and fibrosis of the organs. Ascites and hydrothorax did not exceed 100-150 ml, but the body weight was doubled (to 4.5 ± 0.5 kg) because of edema. The splanchnomorphometric parameters were mainly increased. Microscopic investigation confirmed that this was due to hypertrophy and hyperplasia of cells of the functional parenchyma of these organs, the edema in them, and venous stasis and initial fibrosis. The cardiomorphometric parameters showed an increase of 2-6 times, mainly on account of hypertrophy of C, and consolidation and stabilization of the congestive pulmonary and hepatic hypertension. The number and size of the fuchsinophilic glycolipoprotein granules in the cytoplasm of the neuroendocrinocytes of the arcuate and other hypothalamic nuclei were increased, and this was accompanied by hyperplasia of the cells in the adenohypophysis and in the zona fasciculata and zona reticularis of the adrenal cortex, i.e., definite activation of the structural elements of the hypothalamo-hypophyseo-adrenal system (HHAS). This was accompanied by structural and functional activation of the conducting cells of the sinus and atrioventricular nodes and bundles of His in the conducting system of the heart, starting with hyperplasia and ending with lysis (Figs. 1-3). Thus in the seven rabbits which survived untreated with the model of CHF, there was sufficient pathomorphometric evidence to confirm its presence, together with partial compensation

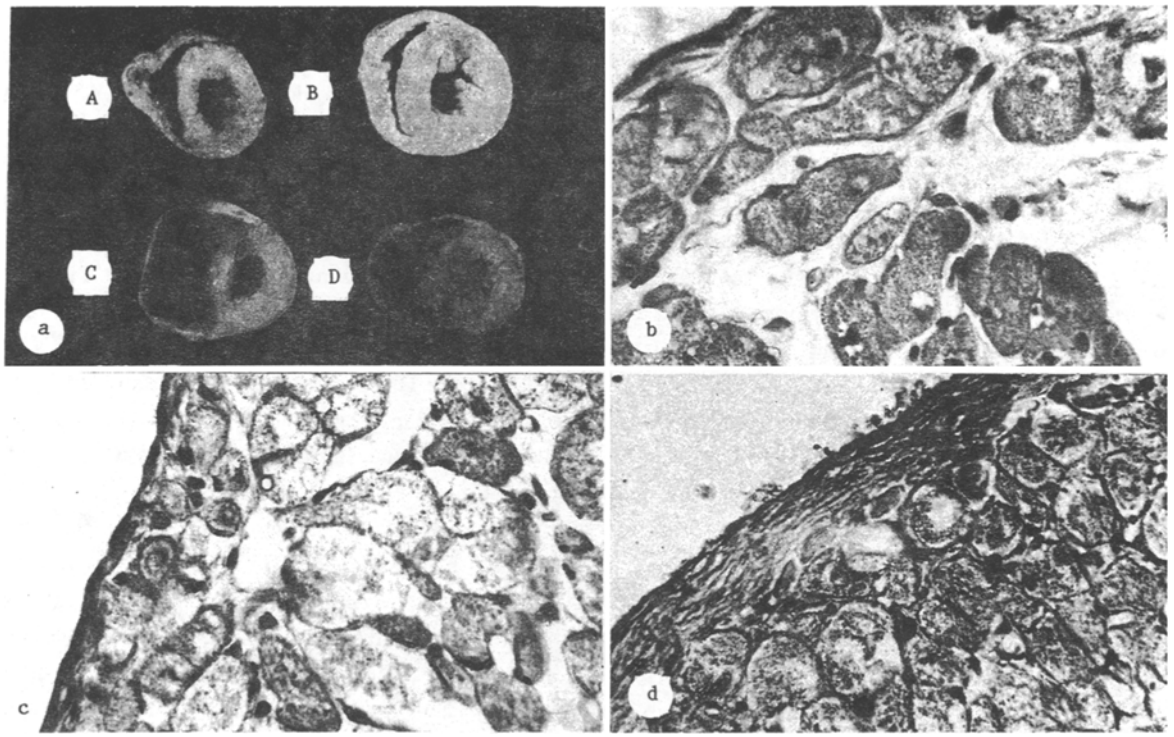

Fig. 1. Pathological criteria of the cardioprotective action of taurine during treatment of experimental CHF in rabbits. a) Macroscopic changes in heart under the influence of treatment: A) control, B) model of CHF with stage II of c.f. (hypertrophy); C) model with treatment of potassium orotate — myocardial hypertrophy and dilatation of ventricles (stage III of c.f.); D) model of CHF with treatment by taurine in a dose of 100 mg/kg — involution of myocardial hypertrophy. Natural size, transverse sections through heart, ×1:1. b, c, d) Changes in conducting system of heart under the influence of taurine treatment, in a dose of 100 mg/kg (microscopic sections stained with hematoxylin and eosin, ×800).

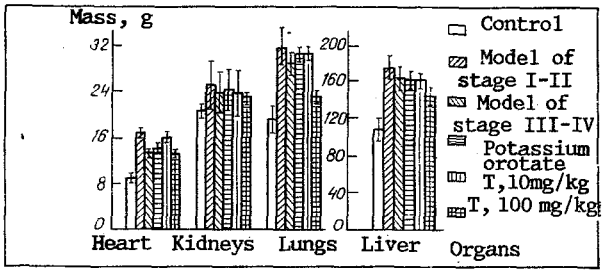

Fig. 2. Splanchnomorphometry of rabbits with CHF under treatment with T.

of stage II of c.f. Group 4) Nine rabbits undergoing the operation, with CHF and stage II-III of c.f., which received 0.5 g of potassium orotate, as the preparation of choice, improving metabolic processes in the myocardium, mainly with their food for 1.5-2 months after the operation. Despite this fact, the CHF progressed in four of them (stage III-IV of CHF) and they died (mortality 44%). The pathological, histochemical, and stereomorphometric features of these rabbits were very similar to those observed during decompensation of the hemodynamics in the untreated rabbits of Group 3 (see Figs. 1, 2, and 3). The tendency for the edema to diminish was not significant, i.e., potassium orotate had no delaying effect on progression of CHF.

In the remaining 18 rabbits the experimental CHF was treated with T, Group 5) Nine rabbits receiving T in a dose of 10 mg/kg daily with the food for 2 months, starting 1 month after aortic valvulotomy and formation of the model of CHF. Of the nine rabbits only one died from CHF and its complications (mortality 11.1%, survival rate 88.9%). In the remaining eight surviving rabbits morbid anatomical, histochemical, and stereomorphometric features of CHF with stage II c.f. were present, very

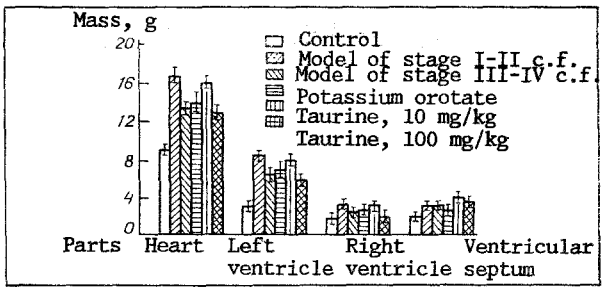

Fig. 3. Differential cardiomorphometry in rabbits with CHF under treatment with T.

similar to those observed in relative compensation of the hemodynamics in Groups 3 and 4 (Fig. 1a). This indicated that T, in a dose of 10 mg/kg, can only prolong the life of the animals, but cannot cure the CHF. Group 6) Nine rabbits with experimental CHF treated for 2 months with T in a dose of 100 mg/kg, i.e., a dose 10 times greater than the previous one. All these animals were alive 3 months after the operation and, judging by the clinical and biochemical tests, they were subjectively better than the other valvulotomized rabbits (survival rate 100%). In particular, signs of improvement of the hemodynamic parameters and ECG were observed. Despite prolongation of the life of these animals, their body weight did not exceed 3.4 ± 0.17 kg and no edema was observed. Pathomorphological, histochemical, and stereomorphometric tests gave results close to the control (Fig. 1), but not identical with it. This normalization was observed in all the activated systems and organs (HHAS, conducting system of the heart, C, and L of the blood vessels), evidence that these doses of T act not only directly on the cardiovascular system, but also indirectly through autonomic centers. However, it must not be forgotten that no evidence of reversibility of established sclerotic and atrophic processes could be observed under these circumstances in the disturbed systems and organs. Even under the influence of high doses of T, involution of the adaptive fibroelastosis of the endocardium, of the diffuse cardiosclerosis, and of the portal and pulmonary hypertension could not be observed in the final stage or in cirrhoeie of the liver. Alternation of zones of atrophy and hypertrophy of C was observed in the myocardium, but normalization of the conducting cells in the conducting system of the heart was invariably incomplete (Fig. 1d), although this may have depended also on the limited (3 months) time of observation of the animals. All the above remarks support the view that even if the cardioprotective effect of T in high doses is not limitless, it nevertheless possesses a well defined and specific regulating action on the cardiovascular system in experimental CHF, restoring pathomorphometric parameters to normal after a course of treatment. Even monotherapy with taurine in sufficiently high doses is undoubtedly useful in the treatment of experimental CHF. It is to be hoped that these experiments will prove useful not only for the interpretation of the pathogenetic action of taurine, but also from the practical point of view, for the clinical treatment of forms of CHF refractory to the usual therapy.

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AN EXPERIMENTAL MODEL OF PEMPHIGUS VULGARIS IN GUINEA PIGS

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The purpose of inducing experimental pemphigus vulgaris (PV) in laboratory animals is to study the mechanisms of onset of this severe autoimmune disease, which affects the skin and mucous membranes in man. It is not yet possible to cure PV, and before corticosteroids appeared the mortality was looa. Creation of a model of PV on newborn BALB/*c* mice [5] by passive transfef of the IgG fraction of serum from patients with PV (IgGPV), although confirming the pathogenetic role of autoantibodies in PV, gave no idea about the other pathogenetec factors involved in the genesis of the foremost histopathological feature of the disease, namely acantholysis (separation of cells of the stratum germinativum of the stratified squamous epithelium). This model of pemphigus likewise cannot be accepted as adequate because, with the ending of injection of pemphigus antibodies, the vesicular eruptions on the animal's skin spontaneously disappear and the mice remain alive.

In the investigation described below methods of inducing manifestations of PV in guinea pigs were screened in order to create a model of PV that corresponds to the criteria of reproduction of the autoimmune disease [12].

EXPERIMENTAL METHOD

Experiments were carried out on 146 noninbred guinea pigs weighing 150-250 g and aged 3.5-5 months. There were three series of experiments: in series I the experimental animals were given an intraperitoneal or intradermal injection of IgGPV or of blister fluid from fresh blisters on the skin of patients with PV (BFPV), into which 10^7/ml peripheral blood mononuclears from a previously untreated patient with a severe form of PV, had been injected beforehand; in series II parallel injections were given of IgGPV (intraperitoneally) and of BFPV, containing mononuclears from a patient with PV, intradermally, and in series III injection of IgGPV (intraperitoneally) was combined with intradermal injections of BFPV (containing mononuclears of a patient with PV) after heating to 56°C (30 min), or exhaustion by combined incubation with a preparation of human skin, or treatment with dexamethasone (100 μg/ml) or contrykal (10 antitrypsin units/ml), or with intradermal injections of BFPV, free from mononuclears. The IgG fraction was isolated from the blood serum of patients with PV by the sulfate-rivanol method [1, 3]. The IgG fraction thus obtained GSS "exhausted" with a human liver preparation [4] and lyophilized with a stage of juxtamural freezing. The final protein concentration was 33.2 ± 0.2 mg/ml, Control animals were given an injection of the IgG fraction of healthy human serum and BF from patients with skin burns of the II degree, containing 10^7/ml peripheral blood mononuclears from the same patients.

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