EFFECT OF SANOTENSIN ON THE ULTRASTRUCTURE OF RABBIT HEART MUSCLE CELLS

N. N. Kleimenova and G. A. Drozdova

UDC 612.172.014.2.014.46:615.217.2

A study of left heart function showed that a two-week course of sanotensin reduces the contractility of the left ventricle. Electron-microscopic investigation of heart muscle cells revealed considerable accumulation of glycogen and lipid droplets in the myocytes of the atria and ventricles after a dose of sanotensin of 1 mg/kg. With an increase in the dose to 2 mg/kg the tendency toward the accumulation of glycogen was increased, distinctive "buds" containing only mitochondria and glycogen were formed, and destructive changes occurred in the mitochondria. The results are evidence of a disturbance of the energy metabolism of the muscle cells, which is evidently one cause of the decreased contractility of the heart muscle.

KEY WORDS: sanotensin; ultrastructure of myocardiocytes; glycogen.

Sanotensin [β-(N-azacyclo-octyl)-ethylguanidine sulfate] is an antiadrenergic compound with a selective sympatholytic action. This compound is widely used in clinical practice for the treatment of hypertension. Sanotensin inhibits adrenergic influences on the cardiovascular system, as shown by a decrease in the peripheral resistance and the cardiac output, slowing of the heart beat, and a decrease in the venous pressure [1, 2, 10]. The essential nature of the processes taking place in heart muscle cells during sanotensin treatment has not yet been adequately explained.

The object of this investigation was to study the fine structure of the heart muscle cells during administration of sanotensin.

EXPERIMENTAL

Experiments were carried out on 20 chinchilla rabbits weighing 2-2.5 kg. Sanotensin was injected subcutaneously as a 0.1% solution for 14 days in doses of 1 mg/kg (series I) and 2 mg/kg (series II) daily. In the course of the experiment the contractility of the heart was determined from the maximal pressure developed by the ventricles during isometric contraction with the ascending aorta occluded for 5 sec. The pressure was measured electromanometrically by catheterization of the open heart. The results were compared with those of the control group (30 animals) and subjected to statistical analysis by Student's method. The level of significance for the difference of the means was $P \leq 0.05$. The animals were killed on the 15th day after the first injection of sanotensin by rapid extraction of the heart.

The myocardium of the left atrium and ventricle was used as the material for electron-microscopic investigation. Pieces of tissue measuring 1 mm^3 were fixed in 1% OsO_4 solution by Caulfield's method, dehydrated, and embedded in Araldite. Electron micrographs were obtained on the JEM-100V electron microscope.

RESULTS

Administration of sanotensin for 2 weeks in the therapeutic dose (1 mg/kg) or a double dose led to

Department of Pathological Anatomy, Stomatologic Faculty, Moscow Medical Stomatologic Institute. Department of Pathological Physiology, Patrice Lumumba University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 12, pp. 91-95, December, 1974. Original article submitted January 24, 1974.

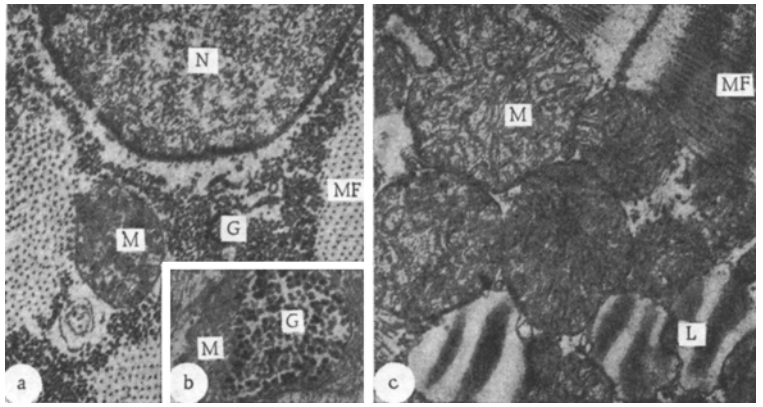

Fig. 1. Myocardium of a rabbit receiving sanotensin in a dose of 1 mg/kg: a) accumulation of glycogen granules in perinuclear zone between myofibrils and mitochondria in muscle cell from the left atrium (22,000 ×); b) glycogen granules inside a mitochondrion (46,000 ×); c) cluster of lipid droplets between mitochondria and myofibrils in muscle cell of the left ventricle (45,000 ×). Here and in Figs. 2 and 3: N) nucleus, G) glycogen, M) mitochondria, MF) myofibrils, L) lipids.

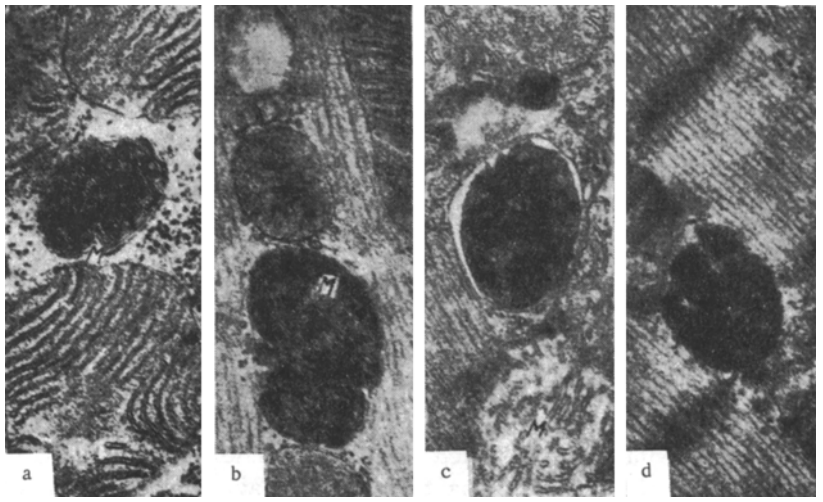

Fig. 2. Myocardium of left ventricle after injection of sanotensin in a dose of 2 mg/kg: a, b, c, d) dynamics of structural changes in mitochondria – accumulation of dense osmiophilic granules on mitochondrial cristae (50,000 ×).

some decrease in the contractility of the left ventricle. The maximal pressure in the control was 238 ± 5 mm Hg, but in the experimental group it fell to 215 ± 9 and 213 ± 8 mm Hg (in series I and II, respectively).

Electron-microscopic investigation of the myocytes from the ventricles and atria of rabbits receiving sanotensin in a dose of 1 mg/kg revealed numerous glycogen granules and lipid droplets. Glycogen granules measuring 300-400 Å, round or polygonal in shape, were distributed diffusely between the myofibrils and myofilaments, sometimes forming large clusters in the perinuclear (Fig. 1a) and subsarcolemmal zones. Sometimes glycogen granules were found in the cisterns of the T-system of the sarcoplasmic reticulum and in the mitochondria (Fig. 1b). The number of micoparticles in the perinuclear zones of the atrial myocytes was reduced, or they were completely absent. As well as the accumulation of glycogen, there was a sharp increase in the content of lipids in the muscle cells of the atria and, in particular, of the ventricles (Fig. 1c). Lipid droplets measuring 0.3-1 μ, with a characteristic periodic alternation of osmophilic density, surrounded by a single membrane, lay in close contact with the mitochondria. The structure of most mitochondria, myofibrils, and nuclei was indistinguishable from normal.

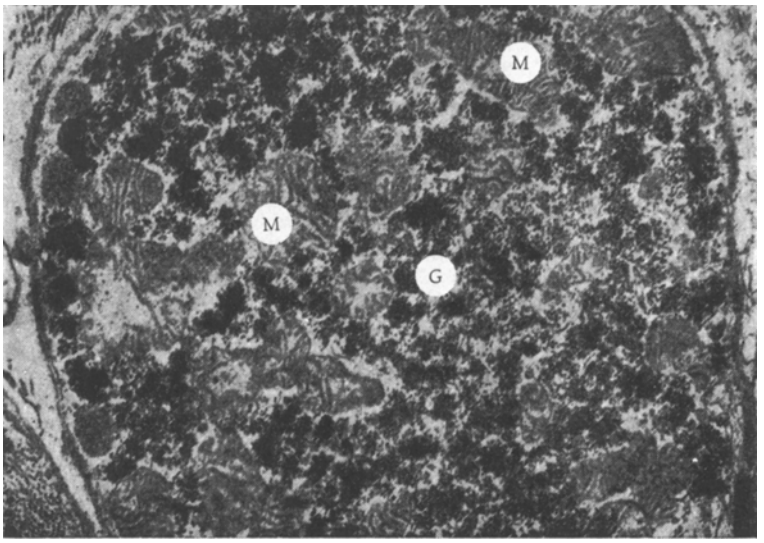

Fig. 3. Myocardium of left ventricle after injection of sanotensin in a dose of 2 mg/kg: evagination of sarcoplasm containing only mitochondria and glycogen granules (18,000 ×).

With an increase in the dose of sanotensin to 2 mg/kg distinctive changes were observed in the structure of the mitochondria and the tendency for glycogen to accumulate was increased. Deposits of floccular osmiophilic material could often be seen on the outer and inner membranes of the mitochondria (Fig. 2a, b, c). Sometimes these mitochondria were surrounded by a single external membrane. The cristae in these mitochondria had lost the clarity of their outlines, they had become osmiophilically dense, and they were fused with the matrix. The mitochondria were converted into structures resembling lysosomes (Fig. 2d). Their size was reduced to 0.5-0.8 μ

The sarcoplasm frequently formed evaginations into the intercellular space containing only mitochondria and glycogen (Fig. 3). Sometimes these distinctive "buds" became detached and lay freely between the muscle cells. Structures of this type were surrounded by a sarcolemma and they measured 30-35 μ in length and 10-12 μ in diameter. Glycogen filling these curious "buds" formed large accumulations resembling bunches of grapes, and the outer membranes of the mitochondria frequently lost their clarity of outline. The structure of the myofibrils and nuclei was indistinguishable from normal.

The electron-microscopic study of the state of the heart muscle cells of the rabbits receiving sanotensin thus showed that this compound has a marked action on muscle tissue. Changes were observed chiefly in the energy metabolism of the cell, as manifested by the accumulation of glycogen and lipids in animals receiving sanotensin in a dose of 1 mg/kg, and in the profound destructive changes in the mitochondria of animals receiving a double dose.

The considerable accumulation of glycogen observed in these experiments in the heart muscle cells was evidently due to the sympatholytic properties of sanotensin, which inhibits both the active transport of amines through the cell membrane and their adsorption in the storage granules [8]. Accordingly, administration of sanotensin brings about a marked decrease in the noradrenalin content in heart muscle [4, 5].

Catecholamines are known to activate adenyl cyclase and this, in turn, leads to the formation of cyclic 3,5-AMP [14-16]. Cyclic 3,5-AMP, through a series of intermediate reactions, converts phosphorylase (a key enzyme in the mobilization of carbohydrates) from the inactive into the active form and thereby intensifies glycogen breakdown [7, 9, 11]. The reduction of adrenergic influences on the myocardium evidently delays glycolytic reactions by disturbing the mechanism of phosphorylase activation. This also can be manifested as the accumulation of glycogen in the muscle cells.

The increase in the lipid content in the myocytes could also be due to a decrease in the catecholamine level in the myocardium. During catecholamine deficiency the utilization of fat is reduced, for adrenalin stimulates the mobilization of nonesterified fatty acids from adipose tissue [3]. The observed decrease in the number of microparticles in the perinuclear zone of the atrial myocytes is indirect evidence of a decrease in the catecholamine level in the myocardium. Correlation between the catecholamine content and the number of microparticles in the atrial tissue has been observed by several workers [6, 12, 13].

The structural changes thus revealed in the mitochondria (marked osmiophilia, loss of clarity of the outlines of the cristae) were evidently also connected with changes in the normal energy level in the cell. Similar disturbances of mitochondrial structure were observed by Winborn and Bockman [17] in the parietal cells of the stomach of normal golden hamsters. These workers regard such changes as an expression of catabolic processes in the cell, leading to the transfer of mitochondria into lysosomes. The considerable number of mitochondria with marked structural changes observed in the present experiments together with the abundant accumulation of glycogen could be evidence of intensified processes of natural "aging" and even "dying" of the mitochondria.

The disturbances of the energy state of the muscle cells of the atria and ventricles noted above – the considerable accumulation of glycogen and lipids, changes in the structure of the mitochondria under the influence of sanotensin in the heart of normal rabbits – are evidently one of the causes of the decreased contractility of the heart muscle during the administration of this substance.

LITERATURE CITED

1. G. A. Glezer and N. P. Moskalenko, Kardiologiya, No. 12, 55 (1972).
2. V. A. Kononyachenko, Essential and Symptomatic Hypertension [in Russian], Moscow (1971).
3. A. Cantarow and Z. W. Schepart, Biochemistry, London (1968).
4. R. Gass, R. Kuntzman, and B. B. Brodie, Proc. Soc. Exp. Biol. (New York), 103, 871 (1960).
5. C. C. Chang, E. Costa, and B. B. Brodie, J. Pharmacol. Exp. Ther., 147, 303 (1965).
6. M. A. Klauda, Proc. Soc. Exp. Biol. (New York), 112, 728 (1963).
7. W. R. Kukovetz, M. A. Hess, Y. Shanfeld, et al., J. Pharmacol., 127, 112 (1959).
8. P. Landborg and R. E. Stitzel, Acta Physiol. Scand., 78, 102 (1968).
9. H. R. Mahler and J. Cordes, in: Basic Biological Chemistry [Russian translation], Moscow (1970), p. 292.
10. N. Page and H. P. Dustan, J. Amer. Med. Ass., 170, 1265 (1959).
11. T. W. Rall, E. W. Sutherland, and S. Berther, J. Biol. Chem., 224, 463 (1957).
12. M. P. Serrano, T. N. Lerdode, L. C. Villanuevey, et al., Arch. Inst. Cardiol. Mexico, 30, 777 (1960).
13. M. R. Sosa-Lucera, F. A. Iglesia, G. Lumb, et al., Lab. Invest., 21, 19 (1969).
14. E. W. Sutherland and T. W. Rall, in: Adrenergic Mechanisms, London (1960), p. 295.
15. E. W. Sutherland, T. W. Rall, and T. Menon, J. Biol. Chem., 237, 1220 (1962).
16. E. W. Sutherland and G. A. Robinson, Pharmacol. Rev., 18, 145 (1966).
17. W. B. Winborn and D. E. Bockman, Lab. Invest., 19, 256 (1968).