ACTION OF STROPHANTHIN K AND β-ACETYLDIGOXIN IN VITRO ON ENERGY TRANSFORMATION BY THE CONTRACTILE PROTEIN SYSTEM OF NORMAL CARDIOMYOCYTES

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KEY WORDS: myocardium; contractile protein system; energy transformation; cardiac glycosides.

It was considered for a long time that cardiac glycosides (CG) act only on the pathological heart, and only if complicated by insufficiency of the myocardial contractile function. However, it has been shown by the study of the action of CG on the intracardiac hemodynamics that they have a positive inotropic action on the normal myocardium also [8].

According to the nowadays generally accepted hypothesis, the positive inotropic effect of GC is due to inhibition of the Na,K-ATPase of the sarcolemma. This gives rise to a number of successive changes in the cardiomyocytes which ultimately lead to strengthening of myocardial contraction [12, 14].

Before this concept developed, on the many hypotheses [7, 13] put forward to explain the mechanism of action of GC there was the hypothesis that it acts on energy transformation by the contractile system of the cardiomyocyte [7]. However this hypothesis was not supported by any reliable experimental evidence.

Experiments on bundles of myocardial fibers with a loosened sarcolemma (which had lost its selective permeability and allowed such large molecules as ATP and CG to pas through freely), with differentially destroyed or functionally inactivated organelles, undertaken previously showed that CG act on intracellular structures of the cardiomyocyte, that β-acetyldigoxin acts directly on the contractile protein system of the myocardium, oaubain and rhodeside act on the sarcoplasmic reticulum, whereas strophanthin K acts on both systems simultaneously [2].

The aim of this investigation was to prove that CG can act on the transformation of chemical energy into mechanical work by the contractile system of the normal cardiomyocyte, and thus to prove the existence of a mechanism regulating energy transformation in this apparatus.

EXPERIMENTAL METHOD

Experiments were carried out on 16 normal chinchilla rabbits weighing 2.5-4 kg. As the isolated contractile protein system of the heart muscle we used bundles of glycerinated myocardial fibers (BGMF), obtained by Szent-Györgyi's method in accordance with the description given for heart muscle [4]. Samples of myocardium were kept in a 50% glucose solution for 1 month, so that only the contractile protein system remained intact in the isolated bundles (0.25-5 mm).

ATP-initiated contraction (final concentration 5 mM) was studied in medium consisting of: 50 mM KCl, 5 mM $MgCl_2$, 20 mM Tris-HCl, pH 8.2, pCa 6.0, at 25°C. To study the action of CG, strophanthin K or β-acetyldigoxin was added to the medium in a concentration of 10^{-6} M. Before contraction the bundles were preincubated for 1 h in the same media.

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TABLE 1. Mechanical and Thermodynamic Parameters of Contraction of BGMF of Normal Heart under the Influence of Strophanthin K and β-Acetyldigoxin (M ± m)

Phase of contraction	Parameter	Control (n = 16)	Strophanthin K (n = 7)	β-Acetyldigoxin (n = 8)
Complete	P, mN/mm^2	4,14±0,41	8,6±0,69*	7,7±1,45*
	$A \cdot 10^{-3}$	1,4±0,37	5,3±1,4*	1,98±0,36**
	ΔH, mJ/mg	206±11,4	394±17,3	287,4±32,3
	ΔQ, mJ/mg	68,3±6,2	78,5±9,2	93,2±6,1*
	ΔH − ΔQ, mJ/mg	144,9±12,2	317,7±18,2*	194,3±33,7**
	$\frac{\Delta H - \Delta Q}{\Delta H} \cdot 100$, %	68,0±3,0	80,0±3,0*	65,0±4,0**
	$\frac{A \cdot 10^{-3}}{A + \Delta Q} \cdot 100$, %	4,3±0,9	9,1±3,5*	2,7±0,3
Generation of force and doing work	ΔH, mJ/mg	177,3±8,6	351,1±26,7	252,3±24,5
	ΔQ, mJ/mg	42,5±3,9	49,2±4,8	60,1±3,4*
	ΔH − ΔQ, mJ/mg	126,5±9,6	302,1±28,8	192,1±23,1
	$\frac{P}{\Delta t}$, $mN/mm^2 \cdot mm$	0,66±0,87	1,32±0,13	1,31±0,18
	$A/\Delta t \cdot 10^{-3}$	0,20±0,05	0,76±0,14	0,44±0,05
	ΔH/Δt	31,2±2,9	53,6±4,8	43,6±4*
	ΔQ/Δt (mJ/mg·min)	7,0±0,6	7,53±0,8	11,2±1,2
	$\frac{\Delta H - \Delta Q}{\Delta t}$ (mJ/mg·min)	23,6±0,6	48,4±3,3*	42,0±9,6*
	$\frac{\Delta H - \Delta Q}{\Delta H} \cdot 100$, %	76,0±1,5	85,0±2,0*	74,0±2,0**
	$\frac{A \cdot 10^{-3}}{A + \Delta Q} \cdot 100$, %	3,0±0,6	12,8±4,0*	4,1±0,3
Maintenance of tension	ΔH/Δt	8,8±1,2	8,0±0,67	7,6±0,4
	ΔQ/Δt (mJ/mg·min)	3,2±0,28	4,7±0,7*	4,8±0,7*
	$\frac{\Delta H - \Delta Q}{\Delta t}$ (mJ/mg·min)	4,1±0,4	3,2±0,6	2,87±0,36
	$\Delta H/F \cdot \Delta t$, mJ/mN·min	2,2±0,2	0,96±0,08	1,0±0,2*
	$\Delta Q/F \cdot \Delta t$, mJ/mN·min	0,78±0,1	0,52±0,07*	0,66±0,14

Legend. *p < 0.05 compared with control; **p < 0.05 between β-acetyldigoxin and strophanthin K groups.

Experiments were carried out by a technique developed previously causing a highly sensitive differential microcalorimeter, resembling in type a Calvet calorimeter, fitted with a strain-gauge device [5]. The strain-gauge spring in these experiments had a coefficient of elasticity of 3.5 M/m, so that the BGMF contracted by 2-3% of the initial length.

Each experiment (on the same material) was repeated 2 or 3 times; their results were averaged and normalized per unit weight of BGMF protein, determined by the micro-Kjeldahl method. The Ca^{++} concentration in the medium was verified with the aid of an F-2110 Ca-selective electrode on an RTS-80 titration system ("Radiometer," Denmark).

The enthalpy (ΔH) liberated by the bundle as a result of hydrolysis of ATP was calculated as the quantity of released inorganic phosphate, determined by Sumner's method [15]. ΔH of hydrolysis of 1 mole ATP in a medium of 20 mM Tris-HCl buffer, pH 8.0, at 25°C was taken to be equal to the sum of ΔH of ATP hydrolysis proper (namely 20.2 kJ/mole [10], and ΔH of neutralization of 1 mole H^+ in Tris-HCl buffer, namely 48 kJ/mole [11].

The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Under the influence of strophanthin K and β-acetyldigoxin, the tension (P) developed by BGMF of the normal heart was doubled or almost doubled relative to the control value, whereas the work done (A) was increased by 4 and 1.4 times respectively (Table 1). The integral amount ΔH of energy released from ATP, real energy which can be used to generate force (F) and to do work with dissipation of a definite amount of heat (ΔQ), was increased by 1.9 and 1.4 times respectively, whereas the integral amount of energy dissipated into heat either remained substantially unchanged, in the case of strophanthin K, or also was increased by 1.7 times, in the case of β-acetyldigoxin. As a result, the integral amount of usefully usable energy (ΔH − ΔQ) and the economy of its use by the contractile protein system of the normal myocardium [(ΔH − ΔQ) × ΔH^{-1}], were substantially increased under the influence of strophanthin K, but unchanged under the influence of β-acetyldigoxin: the mechanical efficiency of the contractile process [A × $(A + \Delta Q)^{-1}$] was increased by 2.1 times under the influence of strophanthin K, but substantially unchanged under the influence of β-acetyldigoxin.

Examination of the thermodynamic parameters of contraction of BGMF during the phases of the contractile process (Table 1) demonstrates clearly that the integral picture of the action of the CG on the mechanical and thermodynamic parameters of contraction of BGMF of the normal heart is determined by their action on processes taking place in the phase of force generation and performance of work – it is in this phase that both the abolute values of ΔH and $\Delta H - \Delta Q$, and also the rates of release of enthalpy ($\Delta H \times \Delta t^{-1}$) and the use of energy by the contractile protein system of the normal myocardium [$(\Delta H - \Delta Q) \times \Delta t^{-1}$], increase. It is in this phase that the absence of any increase in the absolute amount or rate of heat emission in the case of the action of strophanthin K and an increase in these parameters under the influence of acetyldigoxin are clearly recorded.

The phase of maintenance of a state of tension of BGMF of the normal heart (Table 1) compared with the phase of force generation and work performance is characterized by a 3.5-fold decrease in the rate of release of enthalpy and an almost sixfold decrease in the use of energy by the contractile protein system of the normal myocardium. In this phase of the action of strophanthin K and β-acetyldigoxin it will be noted that the rate of release of enthalpy and the rate of its use by BGMF in the case of both CG fall to the same absolute value as when they were not used. Since the rate of release of enthalpy in the phase of force generation and work performance was increased by the action of strophanthin K and β-acetyldigoxin by 1.7 and 1.4 times, in the phase of maintenance of tension it was reduced not by 3.4 times, but by 6.7 and 6 times, and the rate of use of energy was reduced by 15 and 14.6 times respectively.

The next characteristic phenomenon observed in this phase under the influence of CG was an increase in the rate of heat emission against the background of a small but not insignificant decrease in the rate of energy utilization by the contractile protein system. It will be evident that when CG were used less energy was required to maintain the tensile state of the contractile protein system than in the case without CG. This result can be seen more clearly if the rates of release of enthalpy and heat are calculated per unit of maintained force [$\Delta H \times (F \times \Delta t)^{-1}$ and $\Delta Q \times (F \times \Delta t)^{-1}$ respectively] – both parameters are substantially reduced (Table 1); the economy of the process of maintenance of tension is increased.

Thus significant differences are revealed between the action of strophanthin K and of β-acetyldigoxin on the contractile protein system of the normal myocardium. They are found in the phase of generation of force and performance of work. In this connection it is worth mentioning that in clinical practice the view has become established on the basis of empirical data that the use of strophanthin K is indicated mainly in acute heart failure, developing, for example, in myocardial infarction and characterized by the development of an energy-deficient state of the heart muscle [9], whereas preparations of the digitalis group are indicated in chronic heart failure, in whose mechanism an energy deficit does not arise in the initial period [3].

Our own data provide a theoretical basis for the practical use of strophanthin K and CG of the digitalis group in clinical practice, and they lead to the conclusion that a mechanism regulating the economy of transformation of clinical energy into mechanical work both quantitatively and qualitatively, in much the same way as the economizer of the internal combustion engine, functions in the contractile protein system of the myocardium; if the comparison of the biological engine of the muscles with the internal combustion engine is taken further [1], it must also be accepted that this mechanism responds to both endogenous and exogenous situations. For instance, pathological situations can disturb the work of the regulator (for example, in defects and inflammatory lesions of the myocardium [6]) and may lead to a quantitative and qualitative disturbance of energy transformation – to the working of the biological engine of the cardiomyocyte under wasteful conditions, conditions of low mechanical efficiency (the development of heart failure), or they may switch its work into more economic and highly efficient conditions (such as obtained in the case of drug treatment and may be expected as a result of physical training). The existence of such a regulator opens the way to a goal-directed search for new drugs capable of acting both quantitatively and qualitatively, or only quantitatively, on energy transformation by the contractile system of the cardiomyocyte.

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ACTIVATION OF IMMUNOSORPTION PROPERTIES OF BLOOD BY UV IRRADIATION IN THERAPEUTIC DOSES

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The writers showed previously that UV irradiation (UVI) of human blood in therapeutic doses, such as are used in the treatment of various diseases by autologous transfusions of UV-irradiated blood (AUVIB), induces structural and functional changes in the erythrocyte surface [1, 4-6]. This paper describes an attempt to discover whether these changes in the erythrocyte surface are accompanied by potentiation of the immunosorption properties of Rh^+-blood in relation to anti-Rh_0 (D) antibodies. It is important to obtain data of this kind in connection with the search for ways of making the basic method of treatment of hemolytic disease of the newborn (HDN), namely exchange blood transfusion, more effective. In the treatment of HDN due to an Rh conflict, when the fetal tissues absorb a large quantity of material anti-Rh antibodies, the number of exchange transfusions of Rh^--blood sometimes reaches five or six [2].

EXPERIMENTAL METHOD

Immunosorption properties of 18 specimens of blood from Rh^+-donors, stabilized with "Gluyugitsir" solution, eight specimens of Rh^+ packed red cells (PRC), and two specimens of Rh^- blood and Rh^- PRC were studied. The blood and PRC were treated with UV radiation (254 nm) in the "Izol'da" MD-73M mass produced apparatus intended for AUVIB for therapeutic purposes. A standard therapeutic dose of UVI of the blood (1 D) was used, or half that dose (0.5 D) and two or three times that dose (2 D and 3 D). The same blood samples before UVI

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