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THE EFFECT OF GLUCAGON ON THE HEART MUSCLE: RELATION BETWEEN METABOLIC PROCESSES AND CONTRACTILITY

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Abstract—The effect of glucagon on the contractility of the heart muscle (heart rate, left ventricular pressure, dp/dt_{max}) as well as on the oxidative processes of myocardial mitochondria [respiration, respiratory control index, oxidative phosphorylation] and energy stores in the heart muscle [adenosine-triphosphate, creatine-phosphate, glycogen] of rabbits were studied. Glucagon (in the doses of 0.07 mg/kg of body wt) was administered to animals for 15 min. The parameters of contractility (mainly dp/dt_{max}) increased. However, this effect of glucagon only lasted 15–20 min. The respiration of mitochondria simultaneously increased. It provoked neither damage of the mitochondria nor a deleterious situation in the energy stores of the myocardium. On the basis of these results the following conclusions were drawn: (1) Glucagon has a positive inotropic effect of short duration on the heart muscle. (2) In a parallel manner, it enhances oxidative processes in the myocardium at mitochondrial level. (3) It does not provoke either metabolic disturbances of the mitochondria or a deleterious energy situation in the myocardium, and (4) Glucagon is a favourable cardiotonic drug in the therapy of acute but not of chronic heart failure.

The effect of glucagon on the general glycid and lipid metabolism in the organism is well known. In 1960 its pharmacologic effect on the cardiovascular system was described [3]. Since that time glucagon has been used in clinical cardiology mainly for its positive inotropic effect, however the mechanism of this effect remains unsettled. The opinions of different authors concerning the effect of glucagon on the oxidative processes of the heart muscle [8, 10–12] are divided. Knowledge regarding the effect of glucagon on subcellular metabolic processes of myocardium is scarce and requires further studies for explanation of the relation between positive inotropic effect of glucagon on the heart and oxidative and energy yielding processes in the heart muscle. The main purpose of this experimental study was to contribute to this unsettled problem.

MATERIALS AND METHODS

Experiments were performed on rabbits weighing about 2.5 kg (range 2.2–2.8). The experimental group of animals received i.v. injections (U.S.P. Lilly, U.S.A.) in doses of 0.07 mg/kg of body wt dissolved in 15 ml saline solution for 15 min. The control group of animals was given an infusion of saline solution under analogous experimental conditions. During the infusion the animals were under Thiopental anesthesia.

The dynamics of some parameters of myocardial contractility were studied. After *trans*-cutaneous puncture of the left ventricle of the heart, ECG and the pressure in the left ventricle were registered by means of the direct writing ECG apparatus—Mingograf 42 (Elema, Sweden) at a paper speed 100 mm/sec. Simultaneously the dp/dt_{max} of the left ventricle was registered (by means of a derivator of the pressure). All the mentioned parameters were followed at 5, 10, 15, 20, 25 and 30 min intervals from the beginning of the infusion.

For the analysis of the metabolic processes in myocardium the heart was removed from the thorax 15 min after the beginning of the infusion (i.e. after it's termination). The following metabolic parameters were estimated:

(a) Oxidative processes at mitochondrial level. Isolation of the mitochondria from the heart muscle was performed according to Lindenmayer and colleagues [6] in a medium containing EDTA and KCl. The protein content of the mitochondrial suspension was determined according to Lowry and colleagues [7]. The isolated myocardial mitochondria were then used for the determination of their respiration (Q_{02}), respiratory control index (RCI) and oxidative phosphorylation (ADP: O). All the mentioned parameters were analyzed by means of an oxygraph (Gilson Electronics, France) using Clark electrode. The medium in the reaction vessel contained sucrose, Tris-HCl and K_2HPO_4 according to Estabrook [2]. The substrate was glutamate in all experiments.

(b) Content of high-energy phosphates (adenosine-triphosphate and creatine-phosphate) in the myocardium. For these analyses tissue was removed from the opened thorax of the animals using freeze-stop method, Wollenberger's tongs and liquid nitrogen. In the neutralized extract, energy phosphates were determined enzymatically [1].

(c) Glycogen content in the heart muscle. The tissue was solubilized in a KOH solution according

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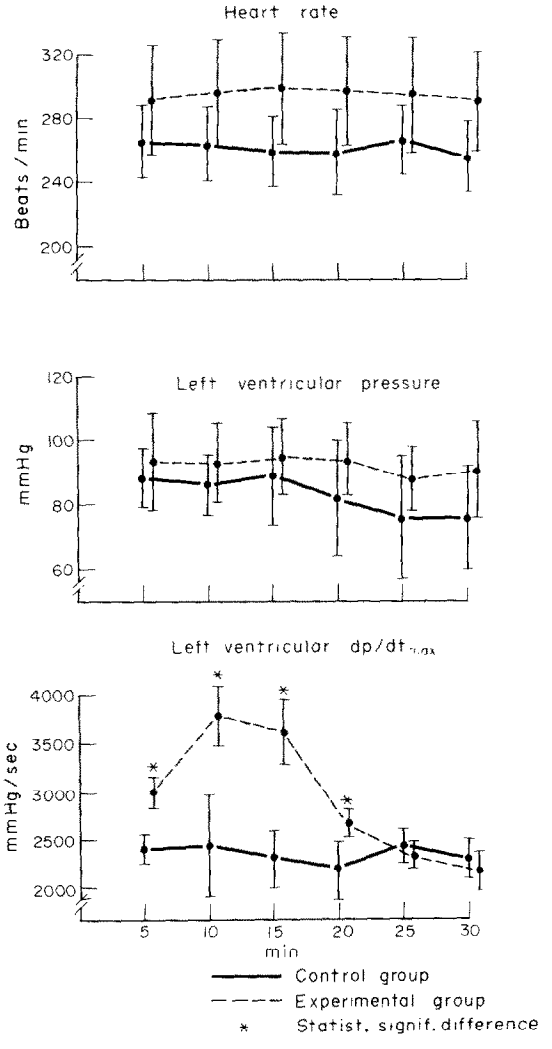

Fig. 1. Heart rate, pressure and dp/dt_{max} of the left ventricle.

to Good and colleagues [4]. Then glycogen was precipitated by ethanol and determined using anthrone reagent according to the method of Morris [9].

All biochemical analyses were performed in duplicate or triplicate parallel samples. The results were evaluated statistically using Student's '*t*' test.

RESULTS

Figure 1 shows that the heart rate of the experimental animals increased by 10–15 per cent (in comparison with the control group of animals). The difference between both groups is not statistically significant (because of large S.D.). This trend lasts during the whole period of observation (30 min).

The same tendency is obvious also in the behaviour of the left ventricle pressure: glucagon infusion provoked an increase of the pressure by 6–19 per cent. This trend remains for 15 min after the infusion.

The increase of dp/dt_{max} of the left ventricle in the experimental group of animals reaches the highest level 10–15 min after the beginning of glucagon infusion (in comparison with the values of control animals it makes 54 and 60 per cent respectively). The difference between both groups is statistically highly significant ($P < 0.001$). However, after the infusion it decreases very rapidly (in the 20 min interval $P < 0.05$) and returns to the values of control animals (25 and 30 min from the beginning of glucagon infusion).

From Table 1 it is obvious that under the influence of glucagon the respiration of isolated myocardial mitochondria (Q_{O_2}) increased by 44 per cent. The difference between the control and experimental group of animals is statistically highly significant ($P < 0.001$). However, respiratory control index of mitochondria (RCI) as well as coefficient of oxidative phosphorylation (ADP: O) estimated at the same time, did not reveal any difference between both groups of animals.

The metabolic situation in the myocardium, namely its energy stores is summarized in Table 2. There is no statistically significant difference between the control and experimental group of animals either in the phosphates (adenosine-triphosphate and creatine-phosphate) or in the glycogen content.

DISCUSSION

Farah and Tuttle [3] established the positive inotropic effect of glucagon in 1960. Since that time it has been repeatedly demonstrated in patients as well as in experimental animals. Nevertheless, there is very little information regarding the effect of glucagon on the metabolic processes of the heart muscle.

Rowe [10] registered an increased extraction of oxygen by the heart muscle in intact dogs. The same effect of glucagon was observed by Moir and Nayler [8] on perfused isolated hearts of dogs. Jesse and colleagues [5] described increased production of CO_2 in the myocardium after glucagon. On the other hand Rudolph and Jehle [11] studied the effect of glucagon (with the doses 1.4–3.5 mg/kg of body wt) in patients on the basis of arterio-coronarovenous

Table 1. Respiration (QO_2), respiratory control index (RCI) and oxidative phosphorylation (ADP : O) in the mitochondria

Statistical parameters	QO_2 (nAtO/mg protein/min)		RCI (nAtO with ADP/n AtO without ADP)		ADP : O (nMol ADP/n AtO)	
	Control	Experiment	Control	Experiment	Control	Experiment
$\bar{x}$	57.6	82.9	8.15	7.58	3.09	3.06
SD	±10.53	±12.38	±3.38	±1.49	±0.11	±0.32
n	11	12	11	12	11	12
P	<0.001		>0.05		>0.05	

Table 2. Content of high-energy phosphates (ATP-adenosine-triphosphate and CP-creatine-phosphate) and glycogen in the myocardium

Statistical parameters	ATP (μmoles/g tissue)		CP (μmoles/g tissue)		Glycogen (μmoles glucose/g tissue)	
	Control	Experiment	Control	Experiment	Control	Experiment
$\bar{x}$	4.83	5.05	4.26	5.96	29.1	26.9
S.D.	±0.62	±1.30	±1.23	±2.63	±4.73	±8.17
n	8	8	8	8	15	19
P	>0.05		>0.05		>0.05	

difference of various substrates and did not find any significant alterations either in the oxygen utilization or in the CO_2 production by the heart muscle.

The aim of our experiments was: (1) The study of glucagon effect on the oxidative processes of the heart muscle at the subcellular level; (2) the study of glucagon effect on the energetic situation of the myocardium, and (3) the correlation of the metabolic alterations with the contractility of the heart muscle.

For this purpose experiments were performed on rabbits using doses of 0.07 mg glucagon/kg of body wt which corresponds to the highest doses used in human cardiology (5 mg/70 kg of body wt).

Under experimental conditions we could observe positive inotropic effect of glucagon on the heart muscle: increased heart rate, increased pressure in the left ventricle as well as dp/dt_{max}, i.e. maximal velocity of pressure increase during isometric contraction of the heart muscle. The three parameters mentioned are the main factors limiting oxygen consumption by myocardium. Their increase is in close relation with the metabolic alterations at mitochondrial level in experiments: oxygen consumption (Q_{O_2}) by mitochondria increased in parallel to dp/dt_{max} increase. However, no deleterious situation in the heart muscle developed as far as its energy processes were concerned. Production of energy is in close relation with consumption of oxygen by mitochondria, which is due to the increased work of the heart muscle after glucagon infusion. This was verified by the physiological values of the respiratory control index and coefficient of oxidative phosphorylation of mitochondria on the one hand and by normal content of high-energy phosphates (adenosine-triphosphate and creatine-phosphate) as well as of glycogen on the other hand.

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