PHYSIOLOGY

# Function of Invertors in Heart Regulation

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It is known that both the contractile and rhythmic heart functions are determined in many respects by the transport and receptor functions of the myocardial plasma membrane [3]. Recently, cellular synthesis of regulators of the cytoplasmatic membrane state (invertors) has been found to occur under the control of the genome [6,7]. Invertors have been proved to activate $Na^+$ and $K^+$-ATPase, to give rise to the opening of the $K^+$-channels, as well as to cause the hyperpolarization of the plasma membrane.

The purpose of the present study was to analyze the possible role of invertors in the regulation of heart function. A long-time perfused isolated heart is a successful model for a study of the intercardiac regulation mechanisms. Under these conditions numerous intracellular and organ mechanisms maintaining heart function are engaged, among which the adrenergic effects on the cardiomyocyte via the adenylate cyclase system (AC) play an important role.

## MATERIALS AND METHODS

Experiments were carried out on passportized albino rats (males) at the age of 6-10 months. The modified technique [10] was employed. Coronary perfusion was carried out in accordance with Langendorf's method under a constant pressure. The electrogram reflecting heart rate (HR) as well as the curves of the filling pressure of the left ventricle and its first derivative reflecting mechanical myocardial efforts were recorded on a "Nihon Kohden" polygraph (Japan). Based on the graphic material, a complex of power ($P_{max}$ - maximum myocardial pressure), rate (*dP/dt* and *-dP/dt* - rates of the development and drop of the intraventricular pressure, respectively), and temporal (TRM - time of reaching $P_{max}$) indexes was calculated, reflecting the state of the contractile function.

cAMP was isolated according to the modified method [9]. cAMP content was measured by the immunocompetitive binding method using a "Cyclic AMP kit" (Amersham, U.K.)

The heart plasma membranes were isolated according to [4]. To obtain cytosol the supernatant resulting from the initial centrifugation of the heart tissue homogenate in imidazole-EDTA-pyrophosphate buffer was ultracentrifuged at 120,000 g. Supernatant in a volume of 50 µl was added to the incubation medium for AC reaction.

AC activity was tested in the plasma membranes in cell-free system. The incubation medium for enzyme activity testing contained 0.05 M Tris-HCl, 0.001 M EDTA, 0.001M dithiothreitol, 0.005 M theophylline, 0.001 M ATP, 0.01 M KCl, 0.005 M

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$MgCl_2$, pH 7.5 at 37°C. Phosphocreatine 0.02 M and phosphocreatine kinase 1 mg/ml were used as the system for ATP regeneration. The total volume of incubation medium was 0.15 ml. The enzyme activity was evaluated by determining the cAMP increment; cAMP content was measured as described above. For additional study of the properties of the AC system adrenalin (50 μmole) or NaF (10 mmole) were added to the incubation medium. Protein content was measured in accordance with the modified Bradford's method [8].

## RESULTS

During the first 2 h of heart perfusion (Fig. 1, *a*) a marked deterioration of heart function was found, namely a decrease of HR, a drop of both the force and rate of contractions and coronary flow, a TRM increase, and a reliable depression of the T-wave on the ECG from the posterolateral wall of the left ventricle. In addition, the rates of both the rise and fall of the ventricular pressure decreased synchronously to the end of perfusion (75% and 77%, respectively). Based on the results obtained as well as published data [1,2], it may be assumed that the heart function disturbances occurring under conditions of long-time perfusion lead to myocardial contracture of the I-II degree.

Intraperitoneal injection of actinomycin D (AMD), an inhibitor of transcription, at a dose of 50 mg/kg 40 min prior to heart isolation (Fig. 1, *b*) results in a moderate decrease of the initial $P_{max}$ value as compared with the control (without AMD injection). AMD appreciably stabilizes heart function during long-time perfusion. Thus, both the rhythmical and contractile functions decreased reliably less as compared with the control. Cyclohexemide (an inhibitor of ribosomal protein synthesis) caused a similar stabilizing effect at a dose of 50 mg/kg. Therefore, inhibitors of the translation and transcription of genetic information have a peculiar protective effect, preventing the development of myocardial contracture.

It is therefore possible to assume the synthesis under conditions of long-time perfusion of factors of peptide nature, which are able to diminish the disturbances in both the rhythmical and contractile heart functions, and, consequently, to stabilize cardiac activity.

For a study of the mechanism of disturbances in heart function and evaluation of the adrenergic mechanism contribution, we examined the myocardial AC system prior to and at the end of perfusion. The escalation of the disturbances in heart function during the period of long-time perfusion was found to be accompanied by changes in the state of the AC complex. As

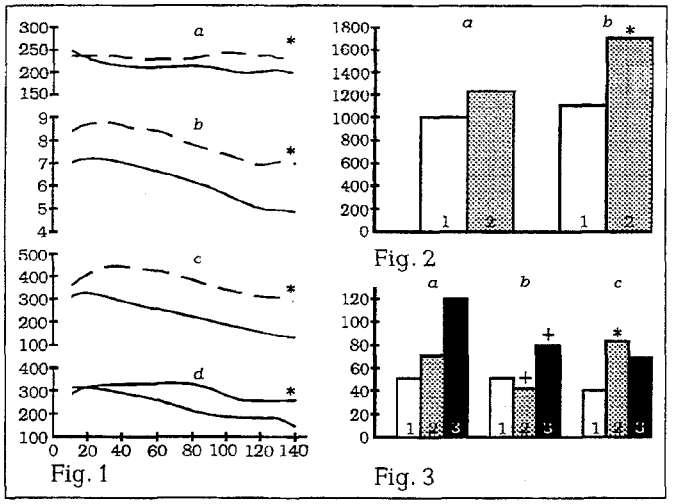


**Fig. 1.** Effect of long−time perfusion and long−time perfusion after actinomycin D treatment on heart rate, beats/min (*a*); $P_{max}$, kPa (*b*); $+dP/dt$, kPa/sec (*c*), and $-dP/dt$, kPa/sec (*d*) of rat isolated heart. *t* − time of perfusion, min; * − differences are reliable.

**Fig. 2.** Effect of long−time perfusion (*a*) and long−time perfusion after actinomycin D treatment (*b*) on cAMP content in myocardium of rat isolated heart. 1) beginning of perfusion; 2) end of perfusion; *: $p<0.05$.

**Fig. 3.** Effect of long−time perfusion and long−time perfusion after actinomycin D treatment on activity of adenylate cyclase from myocardium of rat isolated heart. *a*) beginning of perfusion; *b*) end of perfusion; *c*) end of perfusion after actinomycin D treatment; 1) basal activity of adenylate cyclase; 2) adreno−stimulated activity; 3) fluoride−stimulated activity; +: $P_{2-4}< 0.05$; *: $P_{3-4}<0.05$.

shown in Fig. 2, *a*, no changes in cAMP content or AC basal activity were detected in the first two hours. At the same time, a decrease of both the adreno- and fluoride sensitivity of AC was noted. Since the adrenosensitivity of AC reflects the β-adrenoreceptor state, and the fluoride sensitivity reflects the conjugating function of G-protein with respect to the AC catalytic subunit, then the decline in heart contractile function by the end of perfusion may be associated with changes in these two elements of the AC system.

For a study of the mechanisms of AMD stabilizing effect on heart function, the state of the AC system was tested under the influence of this transcription inhibitor. Prior to the experiment, AMD itself was shown not to affect either the basal or stimulated AC activity of hearts *in vitro* in cell-free system.

During a two-hour perfusion a more significant cAMP content increase induced by AMD was found as compared with the control, which seems to be responsible for a more stable heart rate. At the same time, the AC basal activity practically does not change, while the adrenosensitivity rises markedly in contrast to the experiments without AMD. The AC fluoride sensitivity does not change discernibly and

remains rather lower than in the control. Therefore, AMD prevents the blockade of the AC adrenosensitivity induced by the perfusion disturbances. It may be assumed that AMD affects the receptor-conjugated link. An elevated level of the contractile function of the perfused heart seems to be associated with the stimulation of the adrenergic mechanism due to the suppression of intracellular regulator synthesis.

To confirm the possible presence of factors inhibiting the adrenosensitivity of cells under conditions of long-time perfusion experiments were carried out to create *in vitro* "plasmalemma-cytosol" hybrids. Cytosols from hearts in various functional states, among them cytosols from animals injected preliminary with AMD, were used to ascertain the nature of the factors tested.

The results obtained show that during a direct reaction the control cytosols (14-th of perfusion) do not affect either the basal or stimulated AC activity of the intact heart membrane. At the same time, cytosols from cells of long-time perfused hearts have a weak inhibitory effect on the basal AC activity and a pronounced one on the adrenostimulated activity. Preliminary AMD injection completely eliminates the inhibitory effect of cytosols from cells of the long-time perfused heart both on the basal and adrenostimulated AC activity.

Therefore, the experiments carried out attest to the appearance in cytosols from myocytes of the long-time perfused heart of factors inhibiting the heart adrenosensitivity due to the suppression of AC adrenoreactivity. The inhibitory effect of the transcription inhibitor AMD on their synthesis testifies to their protein (peptide) nature. The findings allow us to conclude that the factors examined belong to the class of invertors - cell regulators, which transmit a control signal from the genome to the nucleus.

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# Role of the Sympathetic and Parasympathetic Nerves in the Development of Vagotomic Tachycardia

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Heart rate acceleration developing after vagus nerve switch off provides evidence that the vagus centers are always in a state of excitation that is termed tone [2]. Changed tone of the vagus nerve may have different effects on the work of the heart, either accelerating or decelerating. The constant tonic efferent activity of the vagus [11] and the development or persistence of developed vagotomic and atropine tachycardias after surgical or drug (propranolol or rausedil) switch off of the sympa-

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